HPLC Analysis of Carotenoids, Tocopherols, and Capsaicinoids of Extracts from Different Peppers (*Capsicum annuum* L.). D.M.A. Molina-Quijada¹, L.A. Medina-Juárez², N. Gámez-Meza², ¹Posgrado en Biociencias. Universidad de Sonora, Hermosillo, Sonora, Mexico., ²Depto. Investigaciones Científicas y Tecnológicas de la Universidad de Sonora, Hermosillo, Sonora, Mexico

This study aims to measure carotenoids, tocopherols, capsaicinoids contents and evaluate antioxidant properties of phenolic extracts from five peppers (Anaheim, Bell, Caribe, Jalapeno and Serrano) by inhibition of cholesterol oxidation, DPPH• and TEAC. Chlorophylls (a and b) were evaluated, beside carotenoids (lutein and β-caroteno). The α-tocopherol content was greater than γ-tocopherol. The highest α-tocopherol content corresponded to Caribe with 779.29±72.48 μg/100 g and the lower was in Jalapeno (282.78±15.19 μg/100 g). The γ-tocopherol content was highest in Anaheim and Caribe, whereas lowest levels were presented in Bell, Serrano and Jalapeno. Differences (p ≤ 0.05) of capsaicin content (Jalapeno>Caribe>Serrano>Anaheim>Bell) were found. The highest level of dihydrocapsaicin (132.88±13.75 μg/g) was found in Bell extract. Bell and Caribe presented the highest antioxidant activity by DPPH• and TEAC. However, phenolic extracts from five pepper showed an inhibition of cholesterol oxidation similar that commercial antioxidant (tocopherol). Therefore, the results show that peppers have potential as a source of natural antioxidants.

Extraction and Analysis of Tomato Seed Oil. F.J. Eller, J.K. Moser, J.A. Kenar, S.L. Taylor, USDA, ARS, NCAUR, Peoria, IL, USA

Tomato seeds represent a very large waste by-product from the processing of tomatoes into products such as tomato juice, sauce and paste. One potential use for these seeds is as a source of vegetable oil. This research investigated the oil content of tomato seeds using several extraction techniques as well as an examination of the oil extracts to determine the composition of the minor constituents such as phytosterol and antioxidant composition. The oxygen radical absorbance capacity (ORAC) of the tomato seed oils were also measured and correlated with antioxidant contents. This research demonstrated that tomato seed oil yield was highest using hot ethanol and followed by hot hexane and finally SC-CO₂. The SC-CO₂ treatment, however, had the highest total phytosterol content as well as highest individual phytosterol content. Sitosterol, cycloartenol, and stigmasterol were the most abundant phytosterols present in the extracts. The highest concentrations of antioxidants were found in the hexane extract. The most abundant antioxidants found in the tomato seed oils were all *trans* lycopene, *cis* 3 lycopene and β-carotene. Oxygen radical absorbance capacity was highest for the hexane extract. Oil yield was inversely proportional to both α-tocopherol and γ-tocopherol content and positively correlated with lycopene *cis* 3 content. ORAC values were positively correlated with only lycopene *cis* 3 demonstrating their role as antioxidants in the tomato seed oil.

Effect of Wavelet Daubechies Denoising on K-Matrix Chemometric Algorithm for the Direct Determination of Lipids in Synthetic Mixtures and Human Serum. Gerard Dumancas¹, Mary Muriuki¹, Neil Purdie¹, Lisa Reilly², ¹Oklahoma State University, Stillwater, OK, USA, ²Bethany College, Bethany, WV, USA

We previously developed a patented reagent system selective to the -CH=CH-CH₂- functional group that can simultaneously quantify the 7 most abundant lipids in human serum that included cholesterol, linoleic, linolenic,
arachidonic, EPA, DHA, and conjugated linoleic using a UV-VIS. Deconvolution of the lipids were done by K-matrix chemometric algorithm using a full factorial design of n=128 training sets and D-optimal design of n=16 validation sets all in synthetic mixtures in chloroform solutions. However, fluctuation of noise is unavoidable during collecting spectra, which will not only worsen the precision of prediction, but also complicate the multivariate model. Wavelet transform was used to eliminate the noise simultaneously in the UV-VIS signals of K-matrix molar absorbance of the 7 lipids and the 16 validation sets. The Daubechies (db) family, 3 threshold selections (Rigrsure, Heursure, and Minimaxi), 3 other threshold selections (one, mln, sln), and different signal levels (j = 1 to j = 4), using a default hard threshold were applied to estimate the performances. Results show that 'db3', 'rigrsure', 'one', 'j = 4'; 'db9', 'rigrsure','one', 'j = 4'; and 'db9', 'minimaxi', 'one', 'j = 4' showed the best denoising performance.

Date Palm (Phoenix dactylifera L.) Kernel Oil and Antioxidant Activity of its Kernel Cake Extracts. M.E.S. Mirghani¹, N.A. Kabbashi¹, I.H. Hussein², Y.B. Che Man³, ¹International Islamic University Malaysia, Gombak, Kuala Lumpur, Malaysia, ²National Oilseed Processing Research Institute, U of Gezira, Medani, Sudan, ³Halal Products Institute, Universiti Putra Malaysia, UPM, Serdang, Selangor, Malaysia

The kernels of date palm (Phoenix dactylifera L.) cultivars Barakawi (B) and Gundelah (G) grown Sudan were analyzed for some of their constituents. Values obtained were protein 5.20%, 5.55%; oil 7.42%, 8.10%; fiber 15.32%, 14.57%; and ash 1.20%, 1.24% for B & G cultivars, respectively. Mineral elements in the ash were Ca, 1.03%, 1.05%; Cu, 0.09%, 0.07%; Fe, 1.02%, 0.82%; K, 20.78%, 23.51%; Mg, 4.17%, 5.07%; Mn, 0.06%, 0.07% and Na, 0.87%, 0.80% for B & G, respectively. The characteristics of date palm kernel oil (DPKO) were; AV 1.04, IV 51.3, SV 216.0, and unsaponifiables 0.9% average for both cultivars. GC showed the major unsaturated fatty acid was oleic 44.32%, 46.31% and main saturated was lauric 20.45%, 17.39% for B & G, respectively. Myristic, palmitic, stearic and linolenic acids were also found in both B and G kernel oils. Total phenolic compounds were 1126.0, 1117.0 mg g-1 for B & G, respectively. The antioxidant activity of the methanolic extracts of the kernel cake was studied by means of Î²-carotene/linoleic acid method. Extracts were markedly effective in inhibiting the oxidation of linoleic acid and bleaching of Å¿-carotene compared to control. This study showed the potential of kernels of Phoenix dactylifera as sources of nutrients, Halal cosmetics beside their antioxidant activity.

Optimization of Extraction Parameters: A Critical Step for Accurate Quantification of Bioactive Phytochemicals. D. Luthria, USDA, ARS, USA

The consumption of fruits and vegetables has been correlated with better health and prevention and/or reduction of risk of certain types of cancer and cardiovascular and neurodegenerative diseases. Fruits and vegetables are known to contain wide array of bioactive phytochemicals (vitamins, minerals, carotenoids, tocopherols, phenolic compounds) that are advantageous for human health. A wide array of functional foods, beverages, and dietary supplements with health claims are commercially produced and easily available in food and drug stores. This presentation will provide an overview of issues related to extraction and assay of phenolic phytochemicals from different matrices (foods, herbs, and dietary supplements). It discusses the importance of optimization of different extraction parameters (solvent composition, particle size, temperature, solid-to-solvent ratio, pressure, and number of cycles) for accurate determination of phenolic compounds. A comparison of current (pressurized-liquid, ultrasonic irradiation, and microwave-assisted) and classical (stirring, Soxhlet, shaker, vortex) extraction procedures for phenolic phytochemicals will also be presented. A general protocol will be outlined for optimizing sample preparation procedure. This optimization process will allow accurate quantification of bioactive phenolic phytochemicals that will enable researchers to provide better guidelines on dietary intake levels necessary to achieve the desired health-beneficial effects.

Preparation, Isolation and Determination of Non-conjugated Geometric/positional Isomers of Linoleic Acid. Ali Reza Fardin Kia, Pierluigi Delmonte, Jeanne I. Rader, US Food and Drug Administration, College Park, MD, USA

Non-conjugated geometric/positional isomers of linoleic acid (c9,c12-18:2 fatty acid, FA) are often present in processed foods and partially hydrogenated vegetable oils (PHVO). The quantitation of these FAs is generally limited by the availability of reference materials. This work focuses on the preparation of pure non-conjugated 18:2 FA isomers, their structural characterization, and separation by gas chromatography. A mixture containing positional and
geometric isomers of 18:2 FA was produced by addition of hydrobromic acid to the linoleic acid double bonds, followed by its elimination with hot alkali. Pure positional/geometric isomers of 18:2 FA were isolated by a combination of silver ion HPLC and sub-ambient temperature reversed phase HPLC. FA double bond position/geometry was determined by a combination of GC covalent adduct chemical ionization MS/MS (CACI-MS/MS) using acetonitrile as the chemical ionization reagent and partial hydrogenation of the pure 18:2 FAMEs with hydrazine. Isolated 18:2 FAs were isomerized with p-toluenesulfonic acid (PTSA) to provide a mixture of their geometric isomers. Solutions containing all the geometric isomers of 8,11-, 8,12-, 8,13-, 9,13-, and 10,13-18:2 fatty acids were prepared as reference materials for quantitation of FA in fats and oils.

**Comprehensive Quantitation of Palm Vitamin E Constituents by High Performance Liquid Chromatography.**

Background This optimization study for Tocotrienols (T3) and Tocopherols (TP) quantitation involved both normal-phase (NP) and reverse-phase (RP) liquid chromatography using various columns and isocratic mobile phases. Results NP systems showed elution of the isomeric analytes in order of increasing polarity with separation based on methyl (CH3) substituents on the chromanol moiety. RP systems showed class separation based on the saturation level of the phytol tail. The UV absorption wavelength at 270nm, analyte concentration $\leq 1600$ppm and calibration curves based on full VE analytical standards provided the optimum linearity coefficients, linear range and accuracy respectively for quantifying the concentration of each analytes. Conclusion The NP Si60 column provided separation of palm VE isomers with non-overlapping peaks, separation times of <20 minutes and reproducible purity results. The procedure has been further adapted to quantify VE isomers in serum or animal organs. The detection sensitivity permits the quantitation of $\geq 10$mg/L of palm VE isomers in serum or animal organs.

**Solvent Fractionation for Natural Product Analysis.** Richard E. Carlson, David E. Knowles, Bruce E. Richter, Dionex Corporation, Salt Lake City, UT, USA

Accelerated solvent extraction (ASE®) is widely used in environmental, food, and polymer analyses to increase the efficiency of the sample preparation process. ASE provides true walk-away automated solvent extraction for solid and semi-solid samples while dramatically reducing extraction time and solvent consumption. While the saving of time and solvent are always favorable, the ability to extract analytes efficiently from diverse matrices is paramount. US EPA Method 3545A, Chinese Method GB/T 19649-2005, German Method L00.00-34, as well as ASTM methods D7210 and D7567 all specify ASE methodology. Recent advances in the use of adsorbents in the extraction cell have enhanced the capability of ASE. For example, adsorbents added to the extraction cell can retain a variety of components such as lipids, steroids, and chlorophyll. Extraction selectivity can also be influenced by sequential extractions with solvents of varying polarity; for example, samples can be extracted first with a nonpolar solvent followed by extraction with solvents of increasing polarity. This paper will discuss the use of adsorbents in the extraction cell to produce extracts that can be analyzed without additional sample cleanup steps. Automatic sequential extraction of samples to produce unique fractionation and selectivity in ASE will also be presented.

**Essential Fats vs. Essential Oils.** Marc Schreuder, Young Family Living Farms, Lehi, Utah, USA

**Gas Chromatography and Mass Spectrometric Analysis of Chia Oil Extracts.** J.W. King¹, J. Lay, Jr.², J. Rocha³, M. Maya³, J. Sacramento³, G. Guillermo ⁴, ¹Department of Chemical Engineering, University of Arkansas, Fayetteville, AR, USA, ²Department of Chemistry, University of Arkansas, Fayetteville, AR, USA, ³Universidad Autonoma de Yucatan, Facultad de Ingenieria Y. Ciencias, Merida, Yucatan, Mexico, ⁴Industrias Oleox S.A, Merida, Yucatan, Mexico

The oil derived from chia seeds (*Salvia polystachya*) and its extracts are of value in nutraceutical technology due to the presence in particular of omega-3 and -6 fatty acids. Chia is propagated in both semi-arid and arid lands in Mexico, Central, and South America and is sold commercially for its health benefits as the seed, oil, and encapsulated extract. In this presentation chia seed oil extracted by various methods ? SFE, ASE, and mechanical expression, have been
characterized by GC and GC-MS with respect to their fatty acid composition. Supercritical fluid extraction (SFE) was performed using SC-CO2 as a solvent at 4000 psi and 40oC. Initial studies comparing a chia oil standard and SFE extracts were found to be very similar in composition in comparing results from GC-FID vs. GC/MS analysis. The choice of GC column and its column length is critical to separating the C18:1 and C18:3 fatty acid methyl esters. Comparison of the FAME profiles from the two different methods indicated equivalency between the SFE-extract and chia oil ?standard?, and compared well to reported values in the literature. The residual carbohydrate content of the extracted seed cake, minus the approximately 30 weight percent extracted oil, was also analyzed with respect to its polysaccharide as well as monomeric sugar content as quantified by HPLC methodology with respect to its potential use as a biofuel precursor.

**AFTERNOON**

**ANA 2: Rapid and Non-Destructive Technologies**

Chair(s): K. Ma, Cognis Corporation, USA; and H. Li, Bruker Optics Inc., USA

**New Development of Networked FT-IR method: AOCS Approved Practice for Biodiesel Analysis.** Barbara Stefl, Ching-hui Tseng, Nan Wang, Cognis Corporation, Cincinnati, OH, USA

Networked FT-IR technology has been used efficiently in the biodiesel analysis, to deliver real time quality information to identify the FFA and moisture of in-coming oils, completion of esterification and transesterification reactions and key parameters of the B100 finished product. This presentation will review the challenges in developing robust IR calibrations and review their performance. Recently, AOCS has approved this method as an official practice for total glycerin, free glycerin, moisture, methanol content, cloud point and acid number. In order to maximize the usefulness of this technology by biodiesel producers and distributors, new methods have been evaluated and developed successfully for the networked platform using patented Chingometrics technology. These methods include: cold flow plug point, (CFPP), oxidative stability, cold soak filtration (CSFT), and cetane number for biodiesel and percentage of biodiesel in biodiesel blends. Investigation is also extended to specification analysis for biodiesel blends.

**In vivo Monitoring of Microalgal Oil Production: A Single-cell Biodiesel-mining Approach.** Huawen Wu1, Joanne Volponi1, Seema Singh1,2, 1Sandia National Laboratories, USA, 2Joint Bioenergy Institute, USA

Transforming algal oil into biodiesel requires solving the problems of growing large robust algae populations that produce high fractions of easily-harvested specific fatty acids. Fatty acid composition and production vary between algae and in response to altered environmental conditions. Problems associated with efficient lipid extraction and lack of reliable lipid characterization techniques have so far made it difficult to generate multi-factorial response curves of the important factors mitigating these problems. We have used our unique in-situ imaging capabilities to develop, fundamental, science-based detailed insights into triacylglyceride (TAG) production. We will present our in situ, in vivo and label-free Raman characterization of model algal lipids, extracted algal oil, and most importantly, individual algae. Our study has demonstrated laser-trapping single-cell confocal Raman spectroscopy to provide direct and quantitative information of the oil produced inside the algae. The lipid triggering effect by nitrate starvation is also observed in vivo. Furthermore, quantitative analysis from the Raman spectra can predict the unsaturation and the melting temperature of the oil, which are directly related to the quality of algal biodiesel and very valuable metrics for determining the suitability of oil for transportation fuel application.

**High-throughput Fats and Oils Analyses Using TD-NMR without Sample Preparation and Solvents.** S. Ghosh, X. Tombokan, Bruker Corporation, The Woodlands, TX, USA

Time-domain nuclear magnetic resonance (TD-NMR) is a powerful non-invasive and rapid analytical technique, which allows looking at samples at molecular level using small bench-top or hand-held systems. It can quantify various components e.g. oil, moisture, protein, etc. accurately and precisely. It is also a very good phase sensor, quantifying crystallization kinetics e.g. solid fat content (SFC) or melting profile in fats and oil blends and such. TD-NMR can also directly quantify the droplet size distribution in o/w or w/o emulsions by probing the restricted diffusion. These are true bulk measurements, irrespective of the color, opacity or surface characteristics of the sample.
These measurements take few seconds and samples can be measured as is. Calibrations of TD-NMR instrument is a very short procedure usually involving 3-5 samples. TD-NMR is currently being used widely in fats and oil industry and academia. This presentation will provide a brief description of the TD-NMR technology followed by various well-established applications relevant to fats/oils industry, with real-life examples. We will also share data from the bleeding edge research in this area including, but not limited to, rheo-NMR, correlation and exchange spectroscopy applications, etc.

**Measurement of Conjugated Linoleic Acid in CLA-Rich Soy Oil by ATR-FTIR.** Jeta Kadamne, Vishal Jain, Mohammed Saleh, Andrew Proctor, University of Arkansas, Fayetteville, Arkansas, USA

CLA isomers in CLA-rich oil are currently measured as FAMEs using GC-FID analysis, which is time consuming as it requires approximately 20 minutes for sample preparation and two hours for analysis. Therefore our objective was to develop an ATR-FTIR method to rapidly determine CLA isomers in CLA rich soy oil. Soy oil was photo-irradiated for 24 hours to produce 96 samples of oil containing CLA levels ranging from 0.38-25.11%. Six replicate CLA GC-FID analyses were performed for each oil sample. The ATR-FTIR spectrum of each oil sample was collected using 128 scans. Multivariate analyses were performed to relate the oil spectral intensities to CLA levels using PLS regression analysis by the Unscramble software. The calibration models were full cross validated and used for predicting unknown CLA levels in oil samples that were not included in the calibration model. The technique predicted the levels of total CLA (R²v= 0.97, RMSEV= 1.14); trans, trans CLA isomers (R²v= 0.98, RMSEV= 0.69); total mono trans CLA isomers (R²v= 0.97, RMSEV= 0.27); trans-10, cis-12 CLA (R²v= 0.98, RMSEV= 0.07); trans-9, cis-11 CLA & cis-10, trans-12 CLA (R²v= 0.97, RMSEV= 0.14) and cis-9, trans-11 CLA (R²v= 0.99, RMSEV= 0.07).

**CANCELED** FT-IR Spectroscopy to Study Structures of Protein Based Matrices in Food Applications. L. Chen and Z. Tian, Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada

**Quality Control of Edible Oils Using an Electronic Nose.** J.C. Mifsud, X. Bredzinski, M. Bonnefille, Alpha MOS, Hanover, MD, USA

Purpose Due to their origin, fish oils can bring an undesirable odor / aroma to the final product. Consequently, it is of utmost importance for manufacturers and users to monitor the sensory features of this ingredient. Method Electronic nose instruments are designed for the analysis of volatile compounds. A metal oxide sensor based E-Nose was used to evaluate the olfactory profile of various fish oils and compare them with 2 reference oils of conform quality. The objective of the study was to set-up a quality control model for a rapid assessment of production batches. Results Instrumental measurement showed a high repeatability (better than 2.5%) and a clear differentiation of the oil qualities. A Principal Component Analysis highlighted the similarities and differences between the samples. Based on the good quality references, a quality control chart was set-up and allowed to determine if the blind samples were conform or out of specifications: out of the different samples, only one was found of acceptable quality. Conclusion The electronic nose instrument can bring the ability to rapidly screen a large number of samples for quality control purposes and thus meets the industrials need for high throughput analysis.

**TUESDAY**

**AFTERNOON**

**ANA 3: Mass Spectrometry**

Chair(s): W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA; and M. Holčapek, University of Pardubice, Czech Republic

Ricinoleate, a monohydroxy fatty acid in castor oil, has many industrial uses. Dihydroxy and trihydroxy fatty acids can also be used in industry. We report here the identification of diacylglycerols and triacylglycerols containing trihydroxy fatty acids in castor oil. The C18 HPLC fractions of castor oil were used for mass spectrometry of lithium adducts to identify trihydroxy fatty acids and the acylglycerols containing trihydroxy fatty acids. Two diacylglycerols identified were triOH18:1-diOH18:1 and triOH18:0-OH18:1. Four triacylglycerols identified were triOH18:1-OH18:1-OH18:1, triOH18:0-OH18:1-OH18:1, triOH18:1-OH18:1-diOH18:1 and triOH18:0-OH18:1-diOH18:1. The structures of these two newly identified trihydroxy fatty acids were proposed as 11,12,13-trihydroxy-9-octadecenoic acid and 11,12,13-trihydroxyoctadecanoic acid. The locations of these trihydroxy fatty acids on the glycerol backbone were almost 100% at the sn-1,3 positions. The content of these acylglycerols containing trihydroxy fatty acids was at the levels of about 1% or less in castor oil.

Identification of Volatile Oxidation Products Responsible for Sensory Degradation of Fish Oil Using GCMS.
J.C. Sullivan¹, S.M. Budge¹, M. St-Onge², ¹Dalhousie University, Halifax, Nova Scotia, Canada, ²Ascenta Health Ltd., Dartmouth, Nova Scotia, Canada

The long chain polyunsaturated fatty acids (PUFA) found in fish oil, specifically EPA and DHA, play an important part in human health. These PUFA are highly susceptible of oxidation due to the large number of double bonds they contain. Popular methods to detect oxidation in fish oil show little correlation to sensory characteristics of the oils. Here we monitor fish oil oxidation using both solid phase microextraction (SPME) headspace analysis and sensory analysis. GCMS was used to identify many of the volatile oxidation products. However, tandem MS techniques were necessary to determine a number of unique oxidation products. Such techniques will be described. Preliminary results suggest that there are combinations of oxidaiton products responsible for off-flavours in fish oil.

Suppression of Electrospray Ionization of Glycerophospholipids by their Lyso Derivatives during Normal Phase LC/ESI-MS. A. Kuksis, A. Ravandi, W. Pruzanski, University of Toronto, Toronto, ON, Canada

While detection and structural characterization of various glycerolipid species by different mass spectrometric methods has continued to improve and reach new levels of sophistication, quantification has been lagging behind. This has been due partly to lack of pure standards and partly due to inability to control the relative proportions of solutes in an unknown sample. We have observed significant suppression of both positive and negative ionization of PtdCho, PtdEtn, PtdSer, and PtdIns by their corresponding lyso-derivatives in group IIA, V and X secretory phospholipase A2 digests of plasma lipoproteins during LC/ESI-MS assays using high sample loads. We have confirmed this observation by analysis of synthetic samples containing different relative proportions of the above glycerophospholipids (0.01-0.5 mg/2 ml). The most severe suppression of ionization was observed with lysoPtdCho in the positive ionization mode. It increased with increasing sample load and increased proportion of the lyso compound in the mixture. The suppression was less severe in the negative ion mode. The acidic glycerophospholipids suffered only minimal suppression of ionization by the presence of their lyso derivatives in the analytical mixture.

Targeted Lipid and Fatty Acid Shotgun Profiling of Complex Extracts by NanoESI-infusion. Brigitte Simons¹, Eva Duchoslav¹, Gary Impey², ¹MDS Analytical Technologies, Concord, ON, Canada, ²Applied Biosystems, Concord, ON, Canada

The growth in lipidomics research is uncovering a need for comprehensive workflows for identifying and quantifying lipid species from complex extracts. A preliminary strategy carried out as global shotgun tandem mass spectrometry by direct infusion electrospray ionization uses information dependant MS/MS scanning in both polarities for unknown lipid profiling. The second approach involves multiple lipid-class-specific precursor ion and neutral loss scanning whose resulting spectra can be used directly to identify and characterize lipids and fatty acids. Fully characterizing these lipid components by high quality MS/MS for fatty acid chain length and double bond positioning is a critical step for understanding their molecular functions and subsequently requires complementary analytical techniques. Taking advantage of the speed, selectivity, and sensitivity of hybrid triple quadrupole technology, analysis by direct nanoESI infusion is shown for in depth glycerophospholipid profiling. Lipid species identification and quantitation is carried out using LipidView™ Software enabling post acquisition processing of precursor ion, neutral loss, MRM, and MS/MS.
data via lipid database searching. We present robust targeted and global workflows for the identification and quantitation of lipids in complex extracts.

**HPLC/MS Analysis of Regioisomeric Triacylglycerols.** Michal Holčapek, Miroslav Lisa, Hana Velínská, University of Pardubice, Pardubice, Czech Republic

TGs are characterized by the total carbon number, the type and stereospecific position of FAs and the number, position and configuration of double bonds. FAs are digested in human or animal organism with the assistance of the stereospecific lipases, where fatty acids from sn-1 and sn-3 position are cleaved first yielding 2-monoacylglycerols. The main possibilities of regiospecific analysis of TGs are the following: 1/ silver-ion LC, 2/ different ratios of fragment ions in APCI mass spectra, 3/ enzymatic hydrolysis, 4/ derivatization followed by chiral LC. Silver-ion normal-phase LC provides a superior separation selectivity for lipids differing in the number, position and configuration of double bonds in FA chains including the resolution of TG regioisomers. Our silver-ion LC method with the coupling of 3 columns in the total length of 75 cm and a new hexane-acetonitrile-isopropanol mobile phase provides an excellent regioisomeric resolution. The randomization is used for the generation of regioisomeric TG series. Applications to complex natural samples of plant oils, animal fats and fish oils containing different TG regioisomers will be demonstrated using 1D and off-line 2D-LC, because non-aqueous reversed-phase and silver-ion LC have quite good orthogonality.Acknowledgement - MSM0021627502 (MSMT) and 203/09/0139 (GACR).

**Determination of the Regiospecific Distribution of Fatty Acid Double Bond Positional Isomers in Triacylglycerols of Berry Oils by High-performance Liquid Chromatographic and Mass Spectrometric Methods.** H. Leskinen, J.-P. Suomela, B. Yang, H. Kallio, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

The positional distribution of fatty acids (FA) in triacylglycerols (TAG) is non-random in natural fats and it varies greatly among fats of different origin. The differences in the regiospecific distribution of FA double bond positional isomers α-linolenic acid and γ-linolenic acid as well as vaccenic acid and oleic acid in TAGs of currant seed oils and sea buckthorn pulp oils were determined by high-performance liquid chromatographic (HPLC) and mass spectrometric (MS) methods. The TAGs containing different FA double bond positional isomers were separated by different HPLC methods prior MS and the regioisomer composition analysis was conducted by electrospray ionization tandem mass spectrometric method using ammoniated TAG precursor ions.

**Effects of Antioxidants on Rapeseed Oil Oxidation in Artificial Digestion Model Analyzed by UPLC-MS.** M. Tarvainen, J.-P. Suomela, H. Kallio, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

A fast ultra performance liquid chromatography (UPLC)-electrospray ionization (ESI)-mass spectrometric (MS) method was used for analyzing effects of different antioxidants on rapeseed oil oxidation in an artificial digestion model. The effects of L-ascorbic acid, 6-O-palmitoyl-L-ascorbic acid, 3,5-di-tert-butyl-4-hydroxytoluene (BHT), DL-α-tocopherol, and DL-α-tocopherol acetate, in different combinations, were assessed. A series of oxidized reference compounds were synthesized and used to identify the oxidized free fatty acid (FFA) and glycerolipid species present in the digested samples. Lithium formate was used in the LC eluents to enhance the formation of lithiated adducts of FFAs and glycerolipids. Ionization efficiencies of sodiated adducts and lithiated adducts were compared in positive ionization mode. Lithiated adducts were formed with enhanced efficiency compared to sodiated adducts. Differences in oxidized lipid profiles were detected in the digested samples containing different antioxidants. Also, the formation of large amounts of sn-1(3)-monoacylglycerols after lipolysis was confirmed.

**Dual Parallel Mass Spectrometry for Analysis of Vitamin D in Retail Fortified Orange Juice.** W.C. Byrdwell¹, J. Exler², S.E. Gebhardt², J.M. Harmly¹, J.M. Holden², K.Y. Patterson², K.M. Phillips³, ¹USDA, ARS, BHNRC, Food Composition and Methods Development Lab, Beltsville, MD, USA, ²USDA, ARS, BHNRC, Nutrient Data Laboratory, Beltsville, MD, USA, ³Virginia Polytechnic Institute and State University, Dept. of Biochemistry, Blacksburg, VA, USA
Samples of vitamin D fortified orange juice obtained from retail food stores were analyzed for vitamin D3 content using a method developed by combining the best features of two AOAC officially approved methods. Detection by ultraviolet absorption at 265 nm was compared to detection by selected ion monitoring (SIM) using atmospheric pressure chemical ionization (APCI) mass spectrometry (MS). Furthermore, an ion trap (IT) mass spectrometer was employed in a dual parallel MS arrangement. The method was applied to 33 samples of 3 national orange juice brands and 7 samples of 5 other brands. The levels determined were compared to the label values, which corresponded to FDA allowed levels. All but one brand exceeded the label amounts. Vitamin D3 values ranged from 1.071 μg/100g (43 IU/100g) to 1.663 μg/100g (67 IU/100g), with an average across 55 samples analyzed, including duplicates, of 1.433 ± 0.114 μg/100g (57.31 ± 4.58 IU/100g). The average of the 38 uniquely identified samples, using the averages of duplicate sets, was 1.415 ± 0.122 μg/100g (56.62 ± 4.88 IU/100g), indicating that a typical 8 oz. glass of orange juice provided 3.397 ± 0.293 μg/100g (135.9 ± 11.7 IU/100g) vitamin D3. Only one store brand out of 39 fortified retail samples was labeled as fortified, but found not to contain vitamin D3.

**Lipid Profiling of Biomass Feedstocks Using Rapid Separation LC with Alternative Solvent Systems and Mass Spectrometry.** L. Lopez, M. Tracy, L. Wang, X. Liu, Dionex Corporation, Sunnyvale, CA, USA

Profiling of lipids in biomass feedstocks is critical for the production of quality biodiesel. Determination of carbon chain length and the degree of saturation is key to ensuring high quality biodiesel that delivers optimum performance during engine combustion. Lipid profiling is commonly done by GC with a flame ionization detector (FID), as specified in the ASTM D6584 and EN 14105 methods; however this methodology has several limitations. High-boiling triglycerides often require special inlet types to preclude discrimination which can cause thermal degradation and interfere with accurate the summative mass closure calculations typically done to determine the total composition of microalgal biomass. Recently, HPLC analysis at ambient temperatures with MS detection has emerged as the preferred method for biomass lipid profiling because it is well-suited to analysis of non-volatile components and can handle very complex or dirty sample matrices without clogging of the LC/MS interface. We demonstrate a lipid profiling method based on LC/MS that features short run times, reduced solvent consumption and elimination of chlorinated solvent with the use of a 2.2 μm C18 columns, temperature control and replacement of dichloromethane by alcohol or ester solvents.
Use of NMR Imaging to Determine the Diffusion Coefficient of Water in Bio-based Hydrogels. K. Doll, BOR-NCAUR, ARS, USDA, Peoria, IL, USA

The diffusion of liquid in a hydrogel material is a fundamental property which must be controlled in order to create effective delivery systems for the agricultural and pharmaceutical industries. NMR spectroscopy has been used to determine the diffusion of water and deuterium oxide in a bio-based hydrogel system. Utilizing an imaging experiment and normalizing and fitting the data to a mathematical treatment published by J. Crank, a diffusion constant of 4.8 x 10^-10 m^2s^-1 was uncovered in a well know biobased water absorbing system. This is considerably slower than the value of for free water, 2.4 x 10^-9 m^2s^-1, showing that the release of solutes from this would be appropriate for some applications. A less quantitative experiment utilizing a dye solution shows a similar trend. These experiments give another parameter which must be optimized to help create high value products from agricultural materials.

Extraction of Lipids from Microalgae. E. Ryckebosch, K. Muylaert, I. Foubert, K.U. Leuven Campus Kortrijk, Kortrijk, Belgium

Microalgae are aquatic organisms that can realize a much higher production per square meter than traditional agricultural plants. They have fatty acids often differing from those in higher animal and vegetable organisms. To screen microalgae for these potentially interesting fatty acids, an analytical procedure to extract the lipids and determine the lipid class and fatty acid composition is necessary. In the literature, a multitude of methods has been utilized. The aim of this research was to propose an optimal protocol for lipid analysis of microalgae. Tests on Phaeodactylum tricornutum and/or Parachlorella sp. showed that lyophilization does not cause any changes and can be performed as a first pretreatment. Endogenic lipases do not present problems, thus a treatment to inactivate them is not essential. Addition of antioxidants is not necessary as no changes in fatty acid composition are observed. No clear differences were detected between different pretreatments when extracting total lipids using chloroform/methanol 1:1. The first extraction extracts > 90% of the lipids. When extracting neutral lipids with petroleumether, very clear differences were observed between different pretreatments and the first extraction only extracts 26% to 92% depending on the pretreatment.

Detection of Diacylglycerol Using High Performance Liquid Chromatography-Charged Aerosol Detector. B.K. Beh¹, O.M. Lai¹,², ¹Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor Malaysia, ²Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor Malaysia

Charged aerosol detector (CAD) has been well suited for lipids determination such as triacylglycerols. However, little information is available for detecting diacylglycerols (DAG) species found in plant edible oil. The aim of this work was to develop a detection and quantification method for single-acid-DAG species using reverse phase-high performance liquid chromatography coupled with CAD. Calibration curves of saturated (C10:0 to C18:0) and unsaturated (C18:1, C18:2) single acid-DAG commercial/ synthesized standards were measured. The data for limit of quantification and limit of detection (LOD & LOQ) and reproducibility were obtained.

Direct Determination of Glycidyl Esters and MCPD Esters in Vegetable Oils by LC-TOFMS. T. Haines, M. Collison, Archer Daniels Midland Company, Decatur, IL, USA

Current methods for determination of MCPD and glycidol in vegetable oils are plagued by artifacts caused by the sample preparation. The method described in this presentation permits determination of MCPD esters and glycidyl esters in vegetable oil samples without derivatization or pre-extraction which can generate artifacts. Samples are diluted and separated by reversed phase HPLC, with glycidyl esters and MCPD esters directly determined by time-of-flight mass spectrocropy. The method is highly specific, sensitive, and free from artifacts caused by harsh derivatization.

Fatty Acid Analysis without Prior Fat Extraction in Some Difficult Matrices using Selected Acid Treatments. Sneh D. Bhandari, Jerry Leahy, Silliker Inc., Chicago Heights, IL, USA
A variety of matrices including dairy, meat, flour based and breaded meat samples were analyzed for fatty acids by the AOAC 996.11 as well as the direct methods which do not require prior fat extraction. The performance of different methods were compared. The performance of some of the direct methods were evaluated with and without inclusion of an acid treatment step. Different acid treatment steps were used during the evaluation. Comparative results of performance of different acid steps in fatty acid analysis of these matrices will be presented.

Determination of Mono-, Di-, and Tri-acyl Glycerols in Biodiesel Fuels by HPLC. D.C. Hurum, B.M. De Borba, L.L. Lopez, J.S. Rohrer, Dionex Corporation, Sunnyvale, CA, USA

Profiling of biomass feedstock lipids is critical for the production of quality biodiesel. This analysis is commonly performed by GC/FID (ASTMD6584/EN14105 methods). However, high-boiling triglycerides are prone to thermal degradation, which interferes with mass balance calculations to determine total biomass composition. HPLC analysis with evaporative light-scattering detection (ELSD) is emerging as an alternative method for biomass analysis because it is well-suited to determine components with low volatility compared to the mobile phase used for the separation. The proposed rapid separation method, using an ethyl acetate-acetonitrile gradient on a 2.2 μm Acclaim 120 C18 column, is evaluated for a series of potential acyl glycerols that may be contaminants in biodiesel. Calibration curves for a series of acyl glycerols are demonstrated between 0.015 and 0.15 mg/mL with retention time precisions of <0.07%. Quantification precisions ranged from 1.8-4.9%. Calibration of vegetable oils, potential contaminants in recycled grease biofuels, is demonstrated from 0.025 to 0.15 mg/mL. Biodiesel samples (B99) were spiked with potential contaminants and analyzed for the acyl glycerols. Rapid (10 min) lipid determination in biodiesel fuels based on HPLC-ELSD with simple sample preparation, reduced solvent consumption, and no chlorinated solvent use is demonstrated.

**CANCELED** Development of Biocatalyst Based Microreactors for Lipid Transformations and Blood Profiling. S.M. Mugo and K. Ayton, Grant MacEwan University, Canada

The New Guidance Method for Calibration, Validation and Use of Near Infrared Analyzers. C. Hurburgh¹, B. Igne², ¹Iowa State University, Ames, Iowa, USA, ²Duquesne University, Pittsburgh, PA, USA

AOCS has recently approved a new method, Am 1a-09, Approved 2009, Near Infrared Spectroscopy Instrument Management and Prediction Model Development. This presentation will discuss the key points of the methods and will utilize relevant soybean and soybean meal data from the authors' laboratories to illustrate the importance/application of these steps. Near infrared analyzers can be applied to a wide range of biological materials and many properties with each material. Consistency in approach to calibration and subsequent validation/standardization will reduce inter and intra lab discrepancies, will increase the credibility of results, and will extend the acceptance of near infrared methods in both scientific and commercial venues.

Analysis of Canola Oil by Rapid Analytical Methods (NIR, NMR, FT-NIR). Veronique J. Barthet, Albert Siemens, Canadian Grain Commission, Winnipeg. MB, Canada

The goal of the project is to compare several rapid analytical methods to measure of oil content in canola seed samples. The various apparatus/technologies commercially available used to measure oil content in canola were NIR, FT-NIR and pulse NMR. These various technologies were used in a "non-research" laboratory environment to assess the accuracy, the repeatability and reproducibility of the measurements and to establish if these technologies could be used by the grain industry to measure oil content specifications. Conventional B. napus canola samples covering a range of oil content (35-36 to 49%) and 5 control samples (1 low, 1 high and 3 medium) were sent to several locations. On location, oil content was measured by various methods (3 to 8 instruments per technology/method) in duplicate. Samples were also sent in blind duplicate, this gave 4 results for one sample. Oil content was measured on clean samples and results were reported in % of oil on dry basis. Statistical analyses were performed to determine the repeatability and reproducibility of the various methods/technologies.
New Method for Solid Fat Content (SFC) by TD NMR. Alan Kook\textsuperscript{1}, Christian Tanzer\textsuperscript{2}, \textsuperscript{1}NMR Service + Consulting, Austin, TX USA, \textsuperscript{2}MR Resources, Fitchburg, MA USA

The SFC methodology used by today's TD NMR's is at least 10 years old. To open up the method to a new generation, we propose a revision of the standardization, acquisition and data processing methods to remove the human error associated with the SFC experiment.

Separation of CLA Fatty Acid Isomers from CLA-rich Soy Oil by Reverse Phase Gradient HPLC. Utkarsh Shah, Andrew Proctor, Department of Food Science, University of Arkansas, Fayetteville, AR, USA

Nutritional studies have demonstrated the health benefits of available CLA cis-trans isomers of CLA but until recently no trans-trans CLA isomers have been available to demonstrate their potential health benefit. However, significant nutritional benefits of 20\% CLA rich soy with predominately trans-trans CLA that was obtained photo-isomerization of soy oil. The objective of this study was to demonstrate the separation CLA trans/trans CLA to isolate CLA fatty acids. CLA rich oil was obtained by photo-isomerization of linoleic acid and free fatty acids obtained by saponification and subsequent extraction of fatty acids by partitioning into chloroform in the presence of BHT. 20 ug of these acids in chloroform were then injected into gradient C-18 RP-HPLC with a mobile phase of water/gradient with 0.05\% acetic acid at a flow rate of 1 ml/min. An ELSD detector and PDA detector at 233 nm was used in parallel. CLA conjugated dienes were observed at 233 nm and collected and CLA isomers identified ATR-FTIR. The chromatograms observed by ELSD showed the typical spectra of soy oil with two additional peaks eluting after linoleic acids. The first of the two peaks were identified By ATR-FTIR as containing cis-trans isomers and the second trans-trans isomers. This study showed that CLA free fatty acids can be separated by analytical reverse phase HPLC. We are now anticipate using preparative HPLC to isolate larger qualities of soy CLA isomers for nutrition studies.

AFTERNOON
ANA 5: General Analytical II

Chair(s): T. Mason West, Bunge Oils Inc., USA; and V. Jain, Oil-Dri Corp of America, USA

Texture Measurements: Bridging Qualitative and Quantitative Methodologies. D. Guy, G. Sekosan, T. West, Bunge Oils, Bradley, IL, USA

(1) Why Measure Texture:Traditionally, texture in food processing has been hard to quantify; subjective descriptions such as "plastic" or "brittle" have been used to describe the texture of shortening and terms like "softer" or "firmer" to describe dough or icing, but it has been hard to communicate the exact nature of product texture. (a) Importance to Food Processing (b) Importance to Finished product (2)How Texture has been Measured: Precedent Tools, Instruments and Methods for measuring texture in food products and processes and their shortcomings as field instruments for testing the firmness of food ingredients. (3) The Current Challenge: (a) Create a handheld, field instrument that will: (b) Improve accuracy of measurement (c) Collect data that shows more than the surface texture (d) Yield data in a digital format that is portable (e) Gives consistent results from operator to operator (f) Associate a temperature measurement since so many texture analyses are temperature-dependent (g) Work on a variety of food products (4) A Current Solution: (a) Review new instrument: size, weight, portability, configuration, data collection process, data download to computer and uses of data

Analysis of Triacylglycerols in Fats and Oils by UPLC®-ELSD. K. Ross, Cargill Global Foods Research, Minnetonka, MN, USA

A method has been developed to separate and quantify triacylglycerols (TAG) in fats and oils. It utilizes reversed phase chromatography employing an Agilent Zorbax? XDB-C18 column in conjunction with a Waters® Acquity® UPLC® and evaporative light scattering detection (ELSD). It is an improvement over previous HPLC methods due to its speed, resolution, and reduction in solvent usage. In addition, it utilizes a mobile phase that is compatible with LC-MS allowing the scientist to garner mass spectral data and thus, greater confidence in identifying TAG for which
standards may not exist. This method has been used to analyze samples such as flax seed oil, canola oil, soy bean oil, and other fats and oils. It has also been used to analyze differences in oils due to heating, processing, chemical and/or enzymatic reactions. The method has shown to be robust for major and total TAG in comparison to a soybean oil standard.

**Measuring Moisture and Fat Contents of Breaded Fried Chicken Nuggets Using VIS/NIR Hyper-Spectroscopy.**
A. Yavari¹, M. Hamedi¹, S. Haghbin², ¹Department of Food Technology, Agricultural Biosystem Engineering Faculty, Karaj, Tehran, Iran, ²Food Technology Dept., Agricultural Bioengineering Research Institute, Rasht, Guilan, Iran, ³Food Technology Dept., Agricultural Bioengineering Research Institute, Rasht, Guilan, Iran

Moisture and fat contents are two important parameters in quality evaluation of fried chicken nuggets. This study was undertaken to evaluate moisture and fat contents of fried breaded chicken nuggets using VIS/NIR hyper-spectroscopic technique. Breaded nugget samples were fried for different times in hydrogenated soybean oil in order to obtain various levels of moisture and fat contents. Reflectance spectra of samples were collected within the range of 400-1750 nm using a spectro-radiometer. Partial Least Squares (PLS) calibration models were developed for quantitative evaluation of the two parameters. The R² and Root Mean Square Error (RMSE) for each prediction were calculated to assess the prediction capability of the model. R² values of 0.92 were obtained from cross validation of calibration for total moisture and fat contents. Validation of the calibration resulted in RMSE of 0.105 for moisture content and 0.017 for fat content predictions.

**Chemometric Algorithms for the Direct Determination of Lipids in Human Serum.**
Gerard Dumancas¹, Mary Muriuki¹, Neil Purdie¹, Lisa Reilly², ¹Oklahoma State University, USA, ²Bethany College, USA

Several chemometric models consisting of K-matrix using ordinary least squares (OLS) and non-negative least squares regression (NNLS), ridge regression K-matrix (RR), P-matrix regression (PM), principal component regression (PCR), and partial least squares (PLS) are introduced and applied for the direct determination of lipids in synthetic human serum models. The simple colorimetric assay used is rapid, rugged, inexpensive, and specific to the -C=C-CH₂- group that accomplishes, in a single assay the simultaneous quantitation of cholesterol, omega-3 (methyl esters of linolenic, EPA and DHA) fatty acids, and omega-6 (methyl esters of linoleic, conjugated linoleic, and arachidonic) fatty acids. The principal outcome was that the RR, PM, PCR, and PLS algorithms successfully out-performed the K-matrix approach when applied to the study of synthetic sera in chloroform solutions. In the case of assays for intact human serum specimens, the same PLS model yielded results for omega-3 and omega-6 polyunsaturated fatty acids data that compared very well for the same samples when measured using the gas chromatography-mass spectrometry gold standard method. Similar results were also derived for the between-methods omega-6/omega-3 ratios. In this study therefore, the dominance of PLS over the other chemometric algorithms has been shown.

**Structural Investigation of Stratum Corneum Lipid by Electron Paramagnetic Resonance.**
Kouichi Nakagawa, Fukushima Medical University, Fukushima, Fukushima, Japan

EPR (electron paramagnetic resonance) in conjunction with a slow-tumbling simulation was utilized for defining human stratum corneum (SC) lipid structure. We found that ordering calculated from the simulation is an appropriate index for evaluating SC lipids structure. The SC from two sites (mid-volar forearm and lower-leg) of human volunteers was stripped consecutively from one to three times using a glass plate coated with a cyanoacrylate resin. Aliphatic spin probes, 5-doxylstearic acid (5-DSA) and 3β-doxyl-5α-cholestane (CHL), were used to monitor SC ordering. EPR spectrum of 5-DSA incorporated in the SC demonstrated a characteristic peak for the first strip. However, EPR spectra of CHL in the SC did not show a clear difference for each strip, except for the peak intensity. The results imply that CHL is not incorporated into the lipid phase as easily as is 5-DSA. A slow-tumbling simulation of the EPR spectrum was performed to analyze the detailed lipid structure. The simulation results for 5-DSA show differences in values of the SC ordering as a function of depth. Thus, these results along with the simulation analysis provide detailed SC layer structure.

**Quantitative Method to Measure Glycidol Fatty Acid Esters in Edible Oils.**
Hiroki Shiro¹, Yoshinori Masukawa¹,
Naoki Kondo¹, Naoto Kudo², ¹Tochigi Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai-machi, Haga-gun, Tochigi 321-3497 Japan, ²Tokyo Research Laboratories, Kao Corporation, 2-1-3 Bunka, Sumida-ku, Tokyo 131-8501 Japan

Glycidol fatty acid esters (GEs) in edible oils, mainly consisting of glycidol palmitic, stearic, oleic, linoleic and linolenic acid esters, are supposed to present as food processing contaminants. In response to this, we previously developed a method to quantify those five species of GEs in edible oils in combination with double solid-phase extraction (SPE) and LC-MS. However, that method was limited only to the use of specific fast HPLC and only to the application to liquid oils. Therefore, that method was improved to a more generalized method that uses conventional HPLC and is applicable not only to liquid oils but also to solid ones. LC-MS conditions using conventional HPLC were optimized, and the amount of oil applied to the SPE was then decreased 10-fold (100 → 10 mg) to achieve stable LC-MS measurement even during sequential runs. To expand the applicability to solid oil, the first step of the original SPE procedure was also changed. Collectively, it was demonstrated that this method enables high-sensitively and accurately quantitative detections of GEs in µg ranges per gram of edible oils. The improved method can be fundamentals of a standardized method for the quality control of GEs in edible oils.

SFC Using the Thar PetroAnalyzer for Analysis of Petroleum Distillates and Biodiesel. Curt M. White, TharSFC, USA

Supercritical fluid chromatography (SFC) with flame ionization detection (FID) can be used to determine the olefin content of gasoline using ASTM method D6550 and the aromatic content of diesel fuel with ASTM method D5186. The several states have passed mandates that require that diesel fuel sold there must contain at least 2 weight % biodiesel. We recently extended the application range of the PetroAnalyzer to include several SFC/FID methods for analysis of biodiesel. The first is useful for analysis of B100, and determines the FAME at each carbon number, total FAME, total mono-, di- and triglycerides, free fatty acids and methanol content of B100. This method is useful for analysis of process streams within a biodiesel processing facility, and is useful for quality control of B100 coming into a refinery. The second method determines the biodiesel content of diesel fuel and was originally described by Diehl and DiSanzo. This method is useful to refineries that are required to show that their diesel fuel contains 2 weight % biodiesel and to state regulators testing for compliance.

Analytical Posters

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

Sensory Characterization of Olive Oil Using a Flash GC Electronic Nose.
J.C. Mifsud, X. Bredzinski, M. Bonnefille, Alpha MOS, Hanover, MD, USA

PurposeBeing considered as a healthy ingredient, olive oil is widely consumed, especially in European Mediterranean countries. It is also one of the most regulated food products in Europe and it is classified in 8 grades, based on numerous physico-chemical and sensory parameters. Therefore, for importation purposes, high grades olive oils produced outside Europe, must comply with strict European regulations. Among the quality parameters tested, sensory features are particularly critical.

Method
An ultra fast gas chromatography based Electronic Nose was used to analyze the complex headspace of oil samples, thanks to two capillary columns with different polarities. The objective of the study was to compare and characterize the aroma of ten samples of olive oil produced in America and bound to the European market.

Results
Instrumental analysis showed a clear differentiation of the various oils on a Principal Component Analysis model, with general sensory attributes trends. The analyzer integrated database of Kovats indices allowed to identify the main odorant compounds. Conclusion
The electronic nose can bring the ability to rapidly compare 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 process.

Optimization of trans Fat Determination by Infrared Spectroscopy.
Since trans fat labeling requirements became mandatory in the US and many other countries, there has been an urgent need for accurate analytical methodologies that would facilitate the verification of compliance with the various regulations. The determination of total trans fatty acids by infrared spectroscopy has been a widely used procedure that has been recently validated and standardized as AOCS Official Method Cd 14e-09 in 2009. This negative second derivative attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) official method for the rapid (5 min) determination of total trans fat was further optimized in the present study in order to evaluate the performance of a miniature portable ATR-FTIR system. The C-H out-of-plane deformation infrared band observed at 966 cm\(^{-1}\) is uniquely characteristic of isolated (non-conjugated) double bonds with trans configuration. Official method Cd 14e-09 entails the measurement of the height of the negative second derivative of the trans absorption band. The narrower bandwidths of second derivative features improved sensitivity and accuracy and made it possible to recognize the presence of potential interferences (such as saturated and conjugated fat components) at low trans levels, near 1% (as percent of total fat). Observed data yielded a fully satisfactory signal-to-noise ratio of 25:1. It also indicated that this methodology and instrumentation can be used to rapidly determine the minimum level of trans fat and oil in a product, approximately 1.8% (as percent of total fat), needed to be measured with confidence to meet the declaration requirement of zero trans fat, or 0.5 g trans fat per serving, on the US Nutrition Fact label. Quantitative evaluation of the lower limit of detection will be reported.

**Verification of the Identity of Organic Eggs by Fatty Acid Fingerprinting.**

A. Tres\(^{1,2}\), R. O'Neill\(^1\), M. Rozijn \(^1\), H. van der Kamp \(^1\), M. Alewijn \(^1\), S. van Ruth \(^1\), \(^1\)RIKILT - Institute of Food Safety, Wageningen University and Research Centre, AE Wageningen, The Netherlands, \(^2\)Nutrition and Food Science Department, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

Identifying high value and/or special food products is of great interest for producers, consumers and regulatory authorities. Multivariate statistics allow treatment of a high number of variables together (as is the case of the fatty acid profile) as a fingerprint of food products, in order to obtain models that allow their correct identification. The aim of this study was to develop multivariate statistical models that allow verification of the organic origin of eggs produced in the Netherlands, based on their fatty acid profiles. Sets of organic (24), free range (12) and barn eggs (12), as well as feeds of the laying hen flocks were analyzed for their fatty acid profiles by gas chromatography. Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) were applied to the egg and feed data. Correlations between eggs and feed were sought. Performance of the models was evaluation by cross validation. Egg and feed data sets were also combined, to improve the statistical models. Models allowed the identification of the organic eggs and feed fairly reliably. However, differentiating free range from barn eggs was quite a challenge.

**CANCELED** Quantification of Sugar Esters in Chocolate and Oil Samples.

K. Franke

**CANCELED** Influence of Chemical Refining Process and Oil Type on Bound 3-MCPD Contents.

K. Franke

**Identification and Quantification of Biodiesel and Other Reaction Products by HPLC.**

A.N.A. Aryee\(^1\), L.E. Phillip\(^2\), B.K. Simpson\(^1\), \(^1\)Department of Food Science and Agricultural Chemistry, McGill University (Macdonald Campus), Ste. Anne de Bellevue, QC, Canada, \(^2\)Department of Animal Science, McGill University (Macdonald Campus), Bellevue, QC, Canada

Transesterification of fats and oils via enzymatic or chemical catalysis has been performed for various reasons; such as modification of the functional properties of the oil/fat, or the production of low molecular weight alkyl esters for use as...
biodiesel (BD). Various analytical methods including gas chromatography, high-performance liquid chromatography, gel permeation chromatography, and spectroscopic methods have been used to monitor and characterize the products of transesterification reactions. In this work, a reversed-phase high-performance liquid chromatography (RP-HPLC) method in isocratic elution mode (toluene-acetic acid) was used for the identification and quantification of BD and other reaction intermediates of the transesterification reaction. The size exclusion column (Phenogel™) coupled to the HPLC system simultaneously separated the BD, residual triacylglycerides (TAG), diacylglycerides (DAG), monoacylglycerides (MAG), free fatty acid (FFA) and residual alcohol based on retention time. In a 25 min analytical time and at a flow rate of 1 ml/min, greater than 67% of BD was detected by the HPLC after 48 h of enzyme catalyzed transesterification. Various amounts of TAG (7.47%), DAG (7.84%), MAG (10.62%), FFA (6.59%) compounds were also detected in the course of the reaction. This HPLC method is a sufficiently rapid and convenient technique that requires no derivatization step as compared to the gas chromatography method.

Conformation of Cyclolinopeptides Observed by Circular Dichroism.

Y.Y. Shim1, D.P. Okinyo-Owiti1, P.G. Burnett1, J. Shen1, R. Sammynaiken2, M. Reaney1, 1Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, 2Saskatchewan Structural Sciences Center, University of Saskatchewan, Saskatoon, SK, Canada

Cyclolinopeptides (CLPs) are natural hydrophobic cyclic peptides, which play a defense role in plant seeds. Circular dichroism (CD) spectroscopy shows that the conformational equilibria of these peptides in solution are dominated by unordered structures. The present study investigated the conformation by CD spectroscopy of the CLPs. The conformations of CLP-A, CLP-C, CLP-J, and CLP-K, previously isolated from linseed (Linum usitatissimum), were studied in a solution of aqueous-dimethylsulfoxide (DMSO), as well as in the presence of the most commonly used agent for stabilizing conformation (HEPES). The CD spectra of CLP-A and CLP-C (with methionine sulfoxide, MSO) suggested high levels of α-helices and lower contents of the other secondary structures compared to that of CLP-J and CLP-K. However, overall CLP-J and CLP-K with methionine sulfone (MSN) residue still have >50% of α-helices. These observations were supported by a relatively good fit to the experimental spectra. Oxidation of MSO (CLP-C) to MSN (CLP-K) increases the hydrophilicity and transforms the peptide structure into a stable β-sheet, as compared to the α-helix of MSO. Solutions of CLPs in aqueous-DMSO indicated large amounts of hydrophobic compounds. None of these conformational forms appear to be stabilized by strong intramolecular hydrogen bonds.

Extraction of Fatty Acids from Lipid Pyrolysis Products.

Justice Asomaning, David C. Bressler, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2G6 Canada

Past research developed a novel two-stage pyrolysis technology that converts lipid feedstock to n-alkane/alkene series hydrocarbons which can be used as lubricating oils, solvents and fuels alternatives (1). However, fatty acids of various chain lengths are also formed as part of the pyrolysis products, which are undesirable for such applications. The primary objective of this work was to optimize the extraction of the fatty acids from pyrolysis products using liquid-liquid extraction. Stearic, oleic and linoleic acid pyrolysis products dissolved in toluene were extracted using 0.1M sodium hydroxide in both water and methanol. The aqueous fraction was acidified with 1M hydrochloric acid and back extracted with toluene. Acid number determination and gas chromatography coupled to mass spectrometer was used to determine the extent of extraction of the fatty acids. Results show that extraction with aqueous sodium hydroxide is unsuitable as there is soap formation with no phase separations to effect extraction. Using 0.1M sodium hydroxide in methanol with the addition of water to aid phase separation, resulted in complete extraction of the fatty acids from all the pyrolysis products. This refined extraction process reduced the acid number of the pyrolysate to levels that are acceptable to both industry and regulatory agencies. Reference 1. Maher, K.D., Kirkwood, K.M., Gray, M.R. and Bressler, D.C. (2008). Pyrolytic decarboxylation and cracking of stearic acid. Ind. Eng. Chem. Res. 47(15): 5328-5336.

The Absolute Configurations of Some Hydroxy Fatty Acids from the Royal Jelly of Honeybees (Apis mellifera).

Tetsuya Kodai, Takafumi Nakatani, Naoki Noda, Setsunan University, Hirakata, Osaka, Japan

9-Oxo-2-decenoic acid (9-ODA) is the main semiochemical, for queen recognition and caste maintenance within
honeybee colonies. During the study of the metabolism of 9-ODA by worker bees, Johnston et al. found that 9-ODA was rapidly metabolized to inactive 9-hydroxy derivatives. From these findings, they postulated a pheromone cycle, that is, worker bees could receive 9-ODA from the queen and convert it into inactive optical isomers, 9-hydroxy-2-decenoic acid (9-HDA). The metabolites could then be transported to the worker mandibular glands where it could be passed back to the queen through royal jelly. The queen could then convert it back into an active keto-form by a simple enzymatic reaction. This presumption is supported by the fact that significant amounts of the corresponding 9-HDA present in royal jelly, but no 9-ODA has been so far detected. These information led us expect that royal jelly contain unknown precursors or metabolites, which could be converted into unidentified semiochemicals by the queen. We have made a more detailed survey of a lipid fraction, particularly hydroxy fatty acids, obtained from royal jelly, and have isolated nine hydroxy fatty acids. The $^1$H-NMR spectroscopic data revealed that, similar to the metabolite, 9-HDA ($R:S\ 2:1$), they exist as a mixture of optical or diastereo isomers. The corresponding keto-derivatives were also synthesized.

### 3-MCPD Esters in Edible Oils: Analytical Aspects.
F. Joffre, F. Lacoste, B. Soulet, H. Griffon, ITERG, Pessac, France

3-monochloropropane-1,2 diol (3-MCPD) is a neoformed compound whose content is regulated in acid-hydrolyzed vegetable proteins and soy sauces (EU regulatory limit of 0.02mg per kg). Other food products such as toasts, crackers, bakery products may contain significant amounts of 3-MCPD. Several studies on 3-MCPD formation showed that in heat processed fat containing foodstuffs, 3-MCPD was formed from acylglycerols and chloride ions (1). In refined vegetable oils, significant amounts of fatty acid esterified 3-MCPD are detected, especially in palm oil (2). According to the latest scientific literature, 3-MCPD esters are formed during oil refining, especially the deodorization step. During this step, diacylglycerides can react with chlorides to form 3-MCPD esters. This may be the reason why high levels of 3-MCPD are detected in palm oil. It is still unknown whether 3-MCPD esters have the same toxic effect as free 3-MCPD. One reason is that digestion and bioavailability of 3-MCPD esters have not been completely elucidated. The current method of analysis of 3-MCPD esters is a two-step method. First, a transesterification with sodium methoxide (3) or an acidic hydrolysis with sulphuric acid/methanol (4) is performed. Second, free 3-MCPD formed during the first step is derivatized with phenylboronic acid prior to analysis. Quantification is done using GC/MS with deuterated free 3-MCPD as internal standard. Quantification of 3-MCPD esters in the form of free 3-MCPD does not allow determination of the distribution of the ester forms, although the toxicity of 3-MCPD mono-esters and di-esters may be different from free 3-MCPD toxicity. This is the reason why it is important to develop a method to analyse separately free 3-MCPD, mono-esters of 3-MCPD and di-esters of 3-MCPD. Evaluation of the DGF standard C-III 17b (08) was performed, and the basis of a new method was developed in order to isolate mono- and di-esters of 3-MCPD for further analysis, without any hydrolysis of the ester bond.1. J. Velisek, P. Calta, C. Crews, S. Hasnip, M. Dolezal: 3-Chloropropane-1,2-diol in models simulating processed foods: Precursors and agents causing its decomposition. Czech J Food Sci. 2003, 21, 153?161.2. B. Svejkovska, O. Novotny, V. Divinova, Z. Reblova, M. Dolezal, J. Velisek: Esters of 3-chloropropane-1,2-diol in foodstuffs. Czech J Food Science 2004, 22, 190?196.3. R. Weisshaar: Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. Eur. J. Lipid Sci. Technol. 20084. V. Divinova, B. Svejkovska, M. Dolezal, J. Velisek: Determination of free and bound 3-chloropropane-1,2-diol by gas chromatography with mass spectrometric detection using deuterated 3-chloropropane-1,2-diol as internal standard. Czech J Food Sci. 2004, 22, 182?189.5. DGF Standard Methods C-III 17b (08) - Determination of ester-bound 3-chloropropane-1,2-diol (3-MCPD esters) and 3-MCPD forming substances in fats and oils by means of GC-MS.

### Rapid Method for Lipid Determination of Microalgae.
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Microalgae are exponentially growing aquatic organisms that perform photosynthesis very efficiently. As such, they can realize a much higher production per square meter than traditional agricultural plants. Furthermore, they are poor in structural polysaccharides and richer in proteins and lipids. Microalgae have a characteristic lipid composition, with fatty acids often differing from those in higher animal and vegetable organisms. To screen microalgae for their amount of potentially interesting lipids, an analytical procedure to extract the lipids can be performed. This method is however time- and labor-intensive, making it difficult to screen large numbers of algae. The aim of this research was to propose...
a rapid method for lipid determination of microalgae. Tests were performed on Phaeodactylum tricornutum. The rapid method was developed using Nile Red, a lipid-soluble fluorescent dye. Pretreatment of the algal cell suspensions was avoided as much as possible: it was limited to dilution of the algal suspension till the OD was between 0.1 and 0.3. The area of the fluorescence peak seems to have a much better correlation to the lipid content than fluorescence at one emission wavelength.

**The Unique Benefits of FT-NIR Spectroscopy for At-line and On-line Analysis of Oleochemicals.**
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Near-infrared spectroscopy (NIR) is a powerful technique for qualitative analysis of oleochemicals and quantitative determination of some of their key characteristics like iodine values, acid values, peroxide values, hydroxyl value or moisture content. As an alternative to lengthy laboratory techniques based on sample hydrolysis and titrations, NIR is a non-destructive method that only requires a few seconds per sample and allows to significantly reduce laboratory turn-around time perform. In addition, the use of Fourier-Transform NIR instruments designed with very high reproducibility standards allows a seamless transport of applications developed in a laboratory to industrial oleochemical process, in order to perform real-time on-line process monitoring. Those unique characteristics of NIR spectroscopy will be demonstrated in this poster and illustrated with real industrial data.

**A Simple, One-step Analytical Method for the Chromatographic Analysis of Fatty Acids in Natural Products.**
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Precise fatty acid profiling is an essential step in the characterization of natural products including many food stuffs, a variety of biodiesel processes and clinical diagnostic tests. Because gas chromatography is almost universally used to separate and quantitate fatty acids, it is necessary to isolate and derivatize the acids prior to analysis. This improves the thermal stability of the acids and increases their vapor pressures. The most prevalent method for converting the acids to methyl esters utilizes a methanolic solution of BF₃. However, BF₃ is toxic, is noted for its instability and is not universally available. This work describes a simple method for profiling fatty acids in a complex matrix. The method utilizes reactive pyrolysis (RxPy)-GC which is, actually, thermally assisted hydrolysis and methylation. Microgram quantities of the sample and a few µL of a methanolic solution of an organic alkali are placed in a small sample cup. The cup is then dropped into a µ-furnace (e.g., 350°C) which is interfaced directly to the GC injection port. The fatty acid methyl esters (FAME) are swept onto the GC column. This work will show the FAME profiles of several sample types: oats, switch grass and algae. The impact of using various organic alkalis on the degree of isomerization will be presented. The derivatization and analysis of C22:6 will be used to compare the BF3 and PxPy methods.

**A New Approach Towards Authentication of Animal Fats.**
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This study was carried out approaching new and simple method to identify animal fats and to introduce such knowledge on fatty acids compositions of edible fats and oils. Different types of animal fats had been presented via plotting carbon chains numbers of fatty acids against present of the same fatty acids obtained from the fatty acid profile of each type of animal fat. Animal fats used were obtained from tallow, mutton, Arabian camel, whale blubber, chicken and pig (lard). Significant representative images were shown to different types of animal fats used in this study, which could be used for authentication of a pure type of animal fat. The results of this study provide a new library to authenticate animal fats.

**Determination of Total trans Fat: An Infrared International Collaborative Validation Study.**
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Using infrared (IR) spectroscopy, collaborating scientists in 10 different laboratories measured in duplicate the total trans fat content of 10 fat or oil test samples, two of which were blind duplicates. The procedure used entailed measuring the height of the negative second derivative of the IR absorption band at 966 cm⁻¹. This validated procedure was approved as official method AOCS Cd 14e-09 in 2009. Among the test samples investigated two were collaboratively determined by both IR and by GC, AOCS Ce1h-05; for these same two test samples, IR precision data were found to be consistent with those obtained by GC. The HORRAT value is defined as \[ \frac{\text{Calculated RSD(R)}}{\text{Predicted RSD(R)}} \] and assigned a magnitude of 1.0 with limits of acceptability between 0.5 and 2.0. All the IR data in the present collaborative study yielded HORRAT values within this range, except those obtained for the two blind duplicate lard test samples with overall means of 1.29 and 1.34, respectively, which yielded respective HORRAT values of 2.77 and 3.03. However, a significantly greater HORRAT value of 5.340 was reported by GC (AOCS Ce 1h-05) for this lard test sample with an overall GC mean of 0.9. HORRAT values greater than 2 may indicate several sources of errors including operating below the limit of determination of the method. IR Precision data will be presented and discussed.

**Differences Between the Effects of Microwave and Convection Oven Heating on the Formation of Oxidation Products from Rapeseed Oil.**

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Comparisons of the effects of “ordinary” vs. microwave heating of dietary oils on lipid oxidation and on the formation of lipid oxidation products have been scarce. In this study, rapeseed oil was heated both in convection and microwave ovens so that its average temperature was retained close to 250 °C. The heating time of the oil was 170-190 min. Neither of the heat treatments caused any considerable changes in peroxide value of the oil after a slight initial decrease. \( \rho \)-anisidine value of the oil increased slightly more when it was heated in microwave oven. Oil samples collected at certain time points during the heating were also analyzed by uHPLC-ESI-MS. Interesting differences were found between the ion chromatograms and mass spectra of the samples prepared with the different heating techniques.

**Limitations in the Use of Liquid Chromatography / Mass Spectrometry for the Structural Characterization of DHA Triacylglycerols.**

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The main constituent of edible oils is a complex mixture of triacylglycerol (TAG) isomers in which three fatty acids (FA) are attached to glycerol in the sn-1, sn-2, or sn-3 position. The position of each FA on the glycerol backbone affects the physical properties of the oil as well as nutritional qualities like bioavailability. One approach to determining the identity and location of each fatty acid is the use of liquid chromatography mass spectrometry, because each TAG isomer yields a distinctive mass spectrum. The \( m/z \) value of the diacylglycerol (DAG) fragment ions helps to determine the identity of each FA, while the ratios of the DAG fragment ions can be used to determine the position of each FA on the glycerol backbone. However, the published literature detailing this methodology has been dedicated to vegetable and animal oils that do not contain appreciable amounts docosahexaenoic acid (DHA). In this work, the DAG fragmentation patterns of a series of TAG standards containing unsaturated and mono-unsaturated FA was compared to the DAG fragmentation patterns of TAG standards containing DHA. The results show that the DAG fragment ions generated from DHA containing TAG isomers do not have the expected ratio of DAG signal intensities, and therefore cannot be used to determine the FA positions of DHA containing TAG.

**Characteristics of Palm Oil from Various Sources Using Gas Chromatography and Differential Scanning Calorimetry.**

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Diglycerides (DAGs) are known to influence the crystallization of fats. This study involves evaluation of palm oil and DAG arrangements. These structures include dipalmitin, distearin, and a mixture of palmitin and stearin content as well as total 1,2 and 1,3 DAGs. Gas Chromatography is used to quantify the DAGs. Differential scanning calorimetry (DSC), is also used in the experiment. The GC values of DAG are compared to the enthalpy, melt point, and crystallization temperatures provided by the differential scanning calorimetry (DSC). By evaluating the thermagram, a
correlation between both total DAG content and DAG arrangement and crystallization behavior is established.