Analytical Interest Area
Technical Program Abstracts

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ANA 1: Trace Contaminants

Chairs: K. Hrncirik, Unilever R&D Vlaardingen, The Netherlands;and J. Leigh, US Food & Drug Administration, USA

LC-MS/MS Detection of MCPD and Glycidyl Esters in Infant Formula: Extraction Procedures and Occurrence Studies of Market Infant Formulas. J. Leigh, S. MacMahon, L.S. DeJager, and T.H. Begley, US Food & Drug Administration, USA.

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. A number of studies have reported adverse toxicological effects from these compounds (3-MCPD, 2-MCPD and glycidol) and, therefore, their presence in edible oils (and processed foods containing these oils) is considered a potential health risk. As refined oils serve as the primary fat source in infant formula, there is a need for methodology to detect MCPD and glycidyl esters in infant formula in order to determine levels of exposure.

A validated approach for extracting and detecting MCPD and glycidyl esters from infant formula will be discussed in this presentation. Using an LC-MS/MS method previously developed for the quantitation of MCPD and glycidyl esters in edible oils, extraction efficiencies were calculated for all monoesters, diesters, and glycidyl esters in a homemade infant formula and several spiked commercial formulas. The newly developed method was then applied to the quantitation of these contaminants in commercially available infant formula in the United States.

Towards Standardization of the Analysis of MCPD- and Glycidyl Esters in Food Products—Results of the AOCS Collaborative Study. K. Hrncirik[1], Z. Zelinkova[1], B. Helbling[2], and R.C. Cantrill[2], [1]Unilever, The Netherlands, [2]AOCS, USA.

Over the past years a substantial effort was spent in the development of an analytical methodology for MCPD- and glycidyl esters. Several methods have been compared, validated, and later adopted as AOCS Official Methods (Cd 29a/b/c-12). Whilst the aforementioned methods are suitable solely for the analysis of oils and fats, further attempts have been made to apply these to the analysis of various foodstuffs. However, the analysis of food products is far from being straightforward and a critical perspective is required to avoid false results in food surveys and routine screening. The validation of such methods is further complicated by the lack of a uniform technique for the extraction of these trace contaminants from the food matrix, and the lack of a reference material. Early in 2015, we launched, together with the AOCS, an international collaborative study with the aim of validating a method for the analysis of MCPD- and glycidyl esters in emulsified foods (spreads, mayonnaises). This speech presents detailed results of the study.

3-MCPD and Glycidyl Ester as Process Contaminants in Vegetable Oil Industry—A Practical Approach. A. Creanga[1], A. Radnóti[2], G. Hellner[2], M. Szeliga[3], S. Golinski[2], Z. Kemény[2], and K. Recseg*[2], [1]Bunge, Belgium, [2]Bunge, Hungary, [3]Bunge, Poland.

Food-born process related contaminants require an excessive effort to study their origin, formation mechanism, toxicity, etc. Vegetable oil industry has been facing with the problem of new and undesirable substances, setting higher standards for us, to continuously improve our process, equipment, and control system. 3-MCPD and Glycidyl Esters have been in the focus of interest only in recent years, but tremendous scientific work has been invested. Present study gives an example how the industry react on these challenges.

Comparison of Analytical Methods for the Analysis of MCPD- and Glycidolesters in Respect of Their Feasibility of Automation. F. Campos, H. Fritz, and N. Hinrichsen*, ADM Research GmbH, Germany.

Within the food industry 3-MCPD-, 2-MCPD, and Glycidolesters have become an important issue. Processors of refined vegetable fats made 3-MCPD- and Glycidolester values part of their raw material specifications. Currently, there are three methods that are regarded as reliable for the analysis of these compounds and are also frequently used. These methods are based on the cleavage of the esters and subsequent derivatization and analysis of the free components by GC-MS. The sample preparation is normally time consuming and requires cautiousness of the lab personnel.

For the routine analysis of 3-MCPD- and Glycidolesters, the work group utilized a system that completed the whole sample preparation plus the GC-MS/MS analysis automatically with only a minor input from lab workers or chemists. The paper compares the implemented automated method with the commonly used methods in commercial laboratories.

The DGF-method (AOCS Cd 29c-13) was the easiest to run on an automated system and showed good recovery and reproducibility rates for 3-MCPD- and Glycidolesters. The AOCS Cd 29a-13, and the so called 3-in-1-method (AOCS Cd 29b-13), were more difficult to implement to an automated sample preparation system mostly due to long reaction times in the sample preparation process.

The Frying Process as a Source of Food Contamination by 3-MCPD Esters. A.P. Arisseto[1], P.F.C. Marcolino[2], A.C. Augusti[1], G.R. Scaranelo[1], S.A.G. Berbari[2], A.M.R.O. Miguel[2], M.A. Morgano[2], and E. Vicente[2], [1]State University of Campinas (UNICAMP), Brazil, [2]Food Technology Inst. (ITAL), Brazil.

High concentrations of 3-MCPD esters have been reported in vegetable oils and fats as a result of the refining process. These contaminants represent a public health concern due to the toxic properties of free 3-MCPD, which can be released from its esters by enzymatic hydrolysis in the human gut. Previous investigations showed that commercial fried foods may also contain significant levels of these compounds. To evaluate the conditions in which the frying process could represent a source of contamination by 3-MCPD esters, experiments were conducted using different raw materials (meat, cereals, fruit, bulbs, and tubers) and oils/fats containing different concentrations of 3-MCPD esters. The influence of pre-treatments (blanching and application of edible coatings) was further investigated in potatoes. An in-house validated indirect method based on acid transesterification and GC-MS was used to determine the levels of the compounds. The results indicated that the contamination of fried foods depends on the concentrations of 3-MCPD esters found in the frying medium and may vary according to fat uptake. Blanching increased the contamination up to 33% while the application of pectin reduced by 10% the amounts of 3-MCPD esters in french fries.

Evaluation of 3-MCPDE Formation Under Frying Condition Using Model System. M. Ouchi, A. Sasaki, and M. Shimizu, Global R&D, Health Care Food, Kao Corp., Japan.

3-Monochloro 1,2-propanediol fatty acid esters (3-MCPDE) has been known as process-induced contaminants found in edible oils and oil-related foods. 3-MCPDE is formed during high-temperature refining process or cooking. In this study, we established a new frying model system to investigate 3-MCPDE generation from various oils, including diacylglycerol containing oil under frying condition. The model system showed to be suitable to evaluate the generation of the esters. Effects of frying temperature, time, and adding amount of salt on 3-MCPDE was revealed.

ANA 2: Green Analytical Techniques

Chairs: K. Ma, Eurofins QTA Inc., USA; and Y. Lu, DSM Nutritional Products, USA

Application of Mid-infrared Portable Spectrometery in Determination of Trans-fatty Acid Content in Bakery Products. M. Shotts, M. Plans-Pujolres, and L.E. Rodriguez-Saona, Ohio State University, USA.

Our objective was to develop a predictive model to quantify *trans*-fat levels in bakery and snack products using portable mid-infrared spectrometers. The approach was tested using 35 calibration standards(consisting of trielaidin in triolein and tripalmitin) and 89 test bakery and snack products ranging from 0.5 to 64% *trans*-fat. Fat from test samples was extracted by blending products into powders with liquid nitrogen and extracting the fat via AOAC method #960.39. Spectra were acquired by directly placing the fat (200µL) onto the temperature-controlled (65±1ºC) 5-reflection ZnSe crystal accessory of the portable mid-infrared spectrometer. Linear regression and partial least squares regression (PLSR) models were developed using calibration standards and extracted fats spectra using either the unique absorption signal of the C–H out-of-plane deformation band observed at 966cm^{-1} or the spectral information on the 900-1200cm^{-1} range. Model performance was assessed using a validation set of products. Best model performances were obtained using selected *trans*-fat ranges (0–8% and >8%). PLSR models showed a better fit than the linear models in predicting *trans*-fat levels giving SEP of 0.5 grams of *trans*-fats per 100 grams. Determination of *trans*-fat using portable ATR-MIR spectrometers allows monitoring *trans*-fat levels for compliance and quality control applications.

Utilization of Differential Scanning Calorimetry for Determining the Rise or Depression of Phase Transition Temperatures in Palm Oil and Interesterified Soybean Oil via Lipid-based Additives at Varying Concentrations. A. Milligan, J.J. Tuinstra, and R. Daniels, Stratas Foods, USA.

Differential scanning calorimetry (DSC) thermograms of structure baking fats can provide valuable information on melting characteristics and heats-of-fusion enthalpy (ΔH). Using an unemulsified, reconstituted palm oil and an interesterified soybean oil blend as standard models, four additives that had been identified as high-functioning during previous investigation, were added at concentrations varying from 0.5 to 2%. The effect of these additives were investigated via DSC in concert with scanning electron microscopy (SEM). A selected subset of the results were validated with x-ray diffraction (XRD). DSC thermograms provided insight into onset temperature, energy input, and temporal/thermal length of various phase transitions. Comparing across samples, we were able to determine the degree of modification conferred by the tested additives with respect to phase transition temperature, relative intensity of phase transitions, and phase transition rate. These factors are becoming progressively more important in the production of baking and confectionary fats as the market continues to demand improvement in products aimed at replacing partially hydrogenated fats.

Rapid NMR Determination of Inorganic Cations in Lipid Matrices. Y.B. Monakhova and B.W.K. Diehl*, Spectral Service AG, Germany.

We have recently shown that NMR spectrometry is a useful tool for quantification of important cations in aqueous extracts of food and *Aloe vera* products. The method was further adapted to more complex lipid matrices such as lecithin, soap, detergents, and biodiesel. The sample preparation was optimized by using optimal amounts of three solvents MeOD, $CDCl_3$, and D_2O-CsEDTA to dissolve the samples without phase separation. Therefore, the information about fatty acid distribution and phospholipid profiles can be obtained to estimate common quality-related parameters from the same spectrum. The quantification is based on integration of signals of metal-EDTA complexes at approximately d 2.7ppm for Mg^{2+} and at d 2.5ppm for Ca^{2+}. The method was fully validated regarding specificity, selectivity, reproducibility, and stability.

A Unified Preparatory Method for FAMEs and Sterols in Oilseed Oil and *in situ* Tissue for Subsequent GLC Analyses. T.P. Mawhinney[1,2], R.S. Gitan[2], C.E. Cheadle[2], D.L. Chance[3], J.K. Waters[2], V.V. Mossine[1,2], and Y. Li[2], [1]Biochemistry & Child Health, University of Missouri, USA, [2]Agricultural Experiment Station Chemical Lab., University of Missouri, USA, [3]Molecular Microbiology & Immunology, University of Missouri, USA.

A unique, environmentally-friendly, single-tube, single-step preparatory method is described for oilseed oil and tissue that allows for both the formation and concomitant extraction of fatty acid methyl esters (FAMEs) and free sterols for their subsequent quantitative GLC analyses. Samples, in 4mL methanol containing 4% non-oxidizing catalyst methane sulfonic acid (MSA) and hexane in miscible proportions, are rigorously stirred at varied temperatures and time, chilled, pH adjusted, and hexane partitioned by the addition of cold aqueous sodium gluconate/NaCl. Hexane aliquots are GLC-analyzed for FAMEs directly, and subsequently for sterols as trimethylsilylated derivatives. Complete conversion of oilseed oil and tissue fatty acids to FAMEs is achieved within 120 minutes at 60°C, or 24 hours at ambient. Use of sodium gluconate/NaCl to add polarity to the reaction mixture and adjust its pH, permits FAME and sterol recovery in the now immiscible hexane phase. In conclusion, this FAME and sterol preparatory method employing MSA is eco-friendly, can readily be applied to numerous oilseed oils and tissues, can be performed as a single pot self-extracting reaction, does not produce unwanted byproducts, requires minimal processing steps, and yields quantitative results efficiently,

providing a new tool to address increasing demands for analytical efficiency and environmental conservation.

Rapid Determination of Fat Content in Microbial Fermentation by Time Domain NMR. A.N. Chang, DSM Nutritional Products, USA.

Time Domain NMR (TD-NMR) is used extensively for determination of oil content in seed industry and has demonstrated utility in microalgae. Typically, the method is built using fat content from hexane extraction or nile red staining. Here we demonstrate that TD-NMR Spectroscopy is equivalent to Fatty Acid Methyl Ester (FAME) analysis for determining total fat in microbial biomass and broth samples. This application allows us to monitor and improve microbial fermentation and downstream processing in a timely manner without any hazardous reagents or solvent. Currently, obtaining an accurate measurement of the total lipid in a sample requires harsh saponification followed by esterification to a methyl ester for analysis by Gas Chromatography. Freeze-dried biomass samples from different microorganism containing a range of fat contents (5-60%) samples were analyzed by both the FAME and TD-NMR methods. The data exhibits a strong linear correlation (R^2>0.99) between the methods, thereby allowing rapid determination of total fat in seconds for microbial biomass samples without sample preparation. The fermentation broth can also be measured with another type of pause sequence and the method can be built using OPUS software.

FT-IR and FT-NIR Quality Analysis of Edible Oils and Oilseeds: A Study of Palm Oil and Soybeans. R.J. Packer, PerkinElmer, USA.

Infrared and Near Infrared analysis have grown in popularity in recent years due to the relative ease of use and sampling, as well as lower cost in comparison to chromatographic and mass spec. techniques. Measurements such as free fatty acids, iodine value, and moisture are now commonly carried out by molecular spectroscopy instruments. Whilst the sampling flexibility of near infrared (NIR) allows for analysis of both oil seeds and edible oils, the additional information in mid infrared (MIR) analyses facilitates adulteration and authenticity testing as well. As such these infrared techniques can provide quality and safety measurements in tandem on a wide range of samples. This work highlights the applicability of these techniques to palm oil and soybean analysis.

Fundamentals and Advancements in Analytical SFC. J. Van Antwerp, Waters Corp., USA.

In the past decade, Supercritical Fluid Chromatography (SFC) has demonstrated great promise as the choice of chromatography for chiral compounds, as a general replacement technique for normal phase liquid chromatography (NPLC) and as a general analytical tool for the analysis of lipids. Compared to traditional normal phase HPLC, SFC is on average 3 to 10 times faster and offers alternative selectivity. Using inexpensive CO_2 and a polar modifier such as methanol as the mobile phase, SFC is more cost-effective and environmentally friendly by reducing the consumption and disposal of organic solvents. Widespread adoption of analytical SFC has been hampered by instrumentation which does not perform to the standards established by modern HPLC systems. Advances in technology have brought the performance of modern SFC instrumentation up to the level of modern LC systems.

This talk will describe the advances made to the hardware, software and chemistries that fully exploit the performance of sub 2u chemistries and will show examples of increased performance and robustness as well as applications.

ANA 2.1/LOQ 2b: Prediction of Oxidative Stability, Shelf-life, and Antioxidants Effects

This session developed in conjunction with the Lipid Oxidation and Quality Division
Chairs: H.S. Hwang, USDA, ARS, NCAUR, USA; S.L. Hansen, Cargill, USA; and S. Seegers, Bunge Oils Inc., USA

Quantitative Determination of Antioxidant Distributions in Emulsions: A Partial Solution to the Polar Paradox Problem. L.S. Romsted[1] and C. Bravo-Díaz[2], [1]Rutgers University, USA, [2]University of Vigo, Spain.

Finding a method for determining antioxidant distributions in emulsions has proved difficult. We have demonstrated that pseudophase kinetic models that work in homogeneous microemulsions also work kinetically stable emulsions. Our solution uses a hydrophobic arenediazonium ion probe that reacts with an antioxidant in the interfacial region of emulsions.

The approach works because the totalities of the oil, interfacial, and aqueous can conceptually be treated as separate reaction regions without considering emulsion droplet size, but only the total volumes of the oil, water, and interfacial regions. Because the diffusivities of the reactants in emulsions are near the diffusion-controlled limit, reactants remain in dynamic equilibrium in kinetically stable emulsions after bulk mixing and their concentrations in each region are constant regardless of droplet shape or size.

This talk includes: (a) the assumptions of the pseudophase kinetic model; (b) the properties of the arenediazonium ion probe; and (c) methods for monitoring the reaction. The results demonstrate that our chemical kinetic method provides a versatile and robust approach for determining the effect of emulsion properties on antioxidant distributions and provides a natural explanation for the "cut-off" effect, the drop in antioxidant efficiency with increasing alkyl chain length of a particular antioxidant.

Modified Ferrous Oxidation-xylenol Orange Method to Determine Peroxide Value of Highly Pigmented Oils. R.R. Abuzaytoun[1], S.L. Mackinnon[2], and S.M. Budge[1], [1]Dalhousie University, Canada, [2]National Research Council of Canada (NRC), Canada.

A ferrous oxidation-xylenol orange method (FOX) was modified to measure total peroxides in highly pigmented oils. In the original FOX method, oil hydroperoxides oxidize ferrous ions to ferric ions, which in turn bind to xylenol orange (XO) that has a maximum absorbance at 560nm. When applying the FOX method in this manner, pigments such as carotenoids, which have similar $?_{max}$, will interfere with the absorbance measurement. To solve this problem, a further step was introduced to separate the XO-complex from pigments. In this proposed FOX method, distilled water was added after the XO-complex was formed; the XO-complex was completely extracted into the aqueous layer leaving behind a highly pigmented organic layer. The absorbance of the aqueous layer was measured at 560nm and the lipid hydroperoxide content was determined using a cumene hydroperoxide standard calibration curve. For method validation, oxidized, un-pigmented fish oil was analyzed with both the proposed modified FOX method and AOCS official method for peroxide values. A correlation coefficient of 0.998 was obtained showing that the values determined using the two methods were equivalent. This modified spectrophotometric FOX method for hydroperoxide determination of highly pigmented oils, such as krill and sea cucumber viscera oil, is a valuable alternative to the presently available PV methods.

Comparison of Shelf-life Assessment with Hydroperoxides and Volatile Compounds. J. Liang, F. Niu, D. Lv, Y. Zhang, and Y.R. Jiang*, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., China.

Vegetable oils are susceptible to oxidation because of high content of polyunsaturated fatty acids.

This may bring up unpleasant off-flavor, taste, losses in desirable color and nutritive value, and affect shelf-life consequently. Shelf-life prediction has long been investigated to give industry instruction of storage time. Hydroperoxieds and induction period by accelerated methods have often been used to estimate the shelf-life of oil products with Arrhenius plots. Unfortunately, these studies can hardly reflect the real shelf-life of the products because of complicated factors affect the real shelf-life. In this study, soybean oil, sunflower oil, and corn oil were selected to collect accelerated oxidation data. Hydroperoxies, p-Anisidine value, and several other volatile compounds (hexanal, 2-heptenal, 2-pentyl furan, and 2,4 decadienal) have been tested. The shelf-life was predicted by Arrhenius plots with hydroperoxides and volatile compounds. At the same time, a two-year storage experiment with the same bench of oils was also done to compare the validity of the shelf-life assessments with different indices. These results will be presented.

Rapid Determination of Fryer Oil Quality. M.K. Gupta, MG Edible Oil Consulting, USA.

Fryer oil deteriorates as frying process progresses. As a matter of fact, the oil starts undergoing oxidation as soon as it is heated for frying without doing the actual frying of any food.

Numerous oil quality parameters are measured and reported by various researchers to define the quality level of the oil in the fryer. Among those are FFA (free fatty acid), PV (peroxide value), anisidine value, oxidized fatty acids, polymerized triglycerides, color, viscosity, etc. The buzzword around the frying industry has been TPM (Total Polar Material) in the fryer oil. The pioneering group, DGF (Germany) first suggested that the oil in the fryer is unfit for human consumption. Various countries have followed the

suit and have set their own guidelines for TPM, and some for FFA, to determine the end point of frying oil quality.

In our work, we measured soap by the AOCS Method (Cc-17-95 (09)) and measured TPM by AOCS Method (Cd-20-92 (09)) on oil samples from restaurant fryers and correlated the results. To our astonishment, we found a strong correlation between the two attributes. Therefore, soap in fryer oil can be used for rapid determination of TPM in fryer oil.

Method Development to Predict Frying Oil Stability by Treatment with Various Ingredients. L. Ban, J. Randall, N. Patel, and W.D. Schroeder, Kemin Food Technologies, USA.

This study aims at establishing methods to evaluate the impact of common ingredients on the improvement of frying oil stability, and comparing treated oils of lower stability with untreated oils of higher stability.

Frying oil stability is one of the crucial factors for the quality of both fried goods and oils. A common strategy is to use ingredients that can improve the oxidative stability. However, as frying studies are resource and time intensive, there has not been a study yet that incorporated a large number of ingredients and evaluated their interactions under real frying conditions. A previously developed high-throughput mini-frying method is combined with Design of Experiment to correlate the performance with the combination of ingredients. The variables contained more than 15 ingredients and their combinations were subject to frying in representative commodity oils, high oleic vegetable oils, and partially hydrogenated oils (PHO). The responses included total polar compounds and the production of toxic chemicals like 4-hydroxynonenal. The correlation will be able to aid the investigation to find PHO replacements without compromising oil stability. More importantly, this study will be able to help increase the value of less stable commodity oils which represent the most widely used oils worldwide.

ANA 3: General Analytical

Chair: H. Adams, Archer Daniels Midland Co., USA

What is a Simulacrum and What Does It Tell You About Triacylglycerol Structures? W.C. Byrdwell, USDA, ARS, BHNRC, Food Composition & Methods Development Lab., USA.

A construct called a simulacrum provides all possible solutions to a sum of two mass spectral abundances, based on ratios of those abundances. It is applied to atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) of triacylglycerols (TAG). A simulacrum has precisely defined components, specifically a simulacrum sum, four Possibilities to Observe, two Cases, and eight solutions. A simulacrum with no restrictions is the First General Form of a Simulacrum (FGFS). When one value is specified to be 1 (as in mass spectrometry), the construct is called a Unit Simulacrum (US). When one value is 1 and no value can be greater than 1 (the two specifications dictated by mass spectrometry), the construct is called the Mass Spectrometry Simulacrum (MSS). Simulacra are used with three Critical Ratios calculated from raw abundances to provide structural information about the degree of unsaturation, the identity and quantity of regioisomers, and other structural characteristics. The simulacrum solutions allow the [MH]+ and all diacylglycerol-like fragments, [DAG]+, of TAG to be reproduced from the Critical Ratios. The simulacrum solutions constitute a reduced data set in which more information is provided in fewer values than raw abundances, constituting a compact library of mass spectra.

What Analysis of Triacylglycerol Structures Taught Me About Pi, Space, Mass, and the Periodic Table. W.C. Byrdwell, USDA, ARS, BHNRC, Food Composition & Methods Development Lab., USA.

Presented here is the link between mass spectrometry of triacylglycerols and a new series of equations for space, mass, and the Periodic Table based on a common pattern. The equations incorporate a new understanding of pi, referred to as Whole PI, which solves the "pi symbol paradox". Using Whole PI, the equations for the dimensions of space become C=2(PI)d^p/2p, A=(PI)d^p/2p, and V=(PI)d^p/2p. It is further shown that the second mass, helium, stands in relation to the first mass, hydrogen, the same as the second dimension of space stands in relation to the first dimension of space, specifically, H=2m^p/2p and He=m^p/2p, in which "m" equals the integer unit mass (m=1), the power signifies the atomic number, and the denominator signifies the integer mass of the atom. Because of the similarity to the equations for dimensions of space, the elements may be referred to as dimensions of mass. When arranged in the form of the new equations, the Periodic Table contains exactly ten dimensions of mass, and the other elements can be considered deconstructions of those dimensions of mass. An approximation is introduced for the accurate monoisotopic mass of hydrogen, (PI/3)^(1/6), which is 99.989% accurate to the observed monoisotopic mass of hydrogen.

Advanced Sample Prep Techniques for Total Moisture in Difficult Samples. L.B. Carey, Metrohm, USA Inc., USA.

Karl Fischer titration is recognized as a universal technique for obtaining accurate water content. However, accuracy requires that all moisture, free and bound, is extracted from the sample matrix. Co-solvents, heating, and homogenization are useful techniques for extracting total moisture from difficult samples. In this talk, learn how and when to apply these advanced sample preparation techniques to improve the accuracy of your moisture results.

Separation of Trans-fatty Acids in Human Plasma via Silver Ion Solid Phase Extraction and GC-MS. H.C. Kuiper, N. Wei, L. Zhang, S.L. McGunigale, and H.W. Vesper, Div. of Lab. Sciences, CDC, USA.

Trans-fatty acids (TFA) are positional isomers of naturally occurring cis-fatty acids and are formed industrially via partial hydrogenation of vegetable oils or naturally in ruminant animals, altering the physical properties and biological effects of the fatty acids (FA). TFA intake has been associated with risk factors for cardiovascular disease. However, little is known about the TFA levels in humans, creating the need to assess human TFA concentrations. Many positional and geometric FA isomers have been reported in humans, but some may not be fully separated with current GC methods. This can lead to inaccurate results for certain fatty acids. We employed silver ion solid phase extraction to evaluate the GC resolution of TFA isomers from regular FA in blood using our newly developed GC-MS method. With this procedure, we identified 20 trans fatty acids in blood and found that our new analytical method separates the TFAs palmitelaidic acid, elaidic acid, trans-vaccenic acid, and linoelaidic acid well from cis-FA. Overall, our study found a large number of TFAs in human blood at very low concentrations and confirmed the high specify of our method for palmitelaidic acid, elaidic acid, trans-vaccenic acid, and linoelaidic acid.

Improve Accuracy of Difficult Titrations Using Thermometric Techniques. L.B. Carey, Metrohm, USA Inc., USA.

Titration is considered a fast and economical technique to evaluate a broad scope of analytes. However, getting good results can be challenging when samples are oily, difficult to dissolve, or require indirect measurement. Thermometric titration improves upon this technique by using a sensor that detects small enthalpy change – a universal property of chemical reactions. In this talk, learn how thermometric titration has been used to improve the analysis of sodium, peroxide, phosphate, and bicarbonate in various sample types.

Development and Validation of a LC-MS/MS Method for Analysis of Vitamin K1 (phylloquinone) and K2-7 (Menaquinone) in Food Samples. S.D. Bhandari and T. Gallegos-Peretz, Merieux NutriSciences, Silliker Food Science Center, USA.

A sensitive and specific method for analysis of vitamin K1, as well as vitamin K2-7, was developed and validated in food matrices. The method involves hydrolysis of fat in the sample aliquot by a lipase action followed by solvent extraction of the vitamin. The reconstituted extract is analyzed by LC-MS/MS employing electrospray ionization (ESI) in positive-ion mode and quantified by multiple-reaction monitoring (MRM). Trans-vitamin K1 was separated from the biologically inactive cis-isomer. Isotopic vitamin K1 was used as an internal standard. Vitamins K1 and K2-7 showed a good linear relationship in their response in the evaluated concentration range of 5 to 250ng/mL. The results for vitamin K1 by the method for the NIST SRM 1849a (Infant Adult Nutrition Formula) matched closely with its certified value. The method accuracy in analysis of vitamin K1 was also supported by its satisfactory spike recovery (88-106%) by the method. The method was applied to a wide variety of food matrices for analysis of vitamins K1 and K2-7 with an inter-day precision value in terms of the % RSD in the range of 6-16%. The spike recovery of vitamin K2-7 obtained by the method in food matrices was in the range of 88-102%. The LOQ of the method for analysis of vitamins K1 and K2-7 was 16 and 39ng/g, respectively.

Thermally Activated Microrheology (DWS) for Fat Crystal Analysis. M. Bazin[2], G. Brambilla[1], R. Ramsch[1], M. Fleury[1], M. Vanden Eynden*[2], and M. Meunier[1], [1]Formulaction, France, [2]Formulaction, Inc., USA.

The crystalline form of fats in chocolate, butter, and vegetable oils was studied thanks to microrheology. Passive microrheology studies the mobility and displacement of micron sized particles [1]: we used Multi Speckle Diffusing Wave Spectroscopy (MS-DWS) coupled with an accurate temperature ramp in order to probe the particles displacement to analyze the viscoelastic properties of a given product. Under heating or cooling conditions, particle movements can be related to the crystalline form of the fat: the rearrangements occurring during melting or during crystallization provide crucial data about the fat's polymorphic transitions.

Crystalline form and melting temperature of fats are important data for the elaboration of new products or for quality control of finished products. In the case of chocolate, the microrheology analysis during melting can identify the crystalline form of finished chocolate products, and so help to predict its stability against blooming. Moreover, microrheology can be used to study the impact of formulation and process on melting temperatures of low-fat butters. In addition to the analyses of crystalline forms of fat, the MS-DWS provides data on viscoelastic property changes.

[1] D.A. Weitz, D.J. Pine, in: Dynamic Light Scattering, W. Brown (Ed.) (Oxford Univ. Press, New York, 1993), Chap. 16.

HPLC Determination of Bioactive Compounds in Canola Oil: An Investigation to Enhance Canola Oil Quality. C. Flakelar[1,2], D. Luckett[2,3], J. Howitt[1,4], G. Doran[1,2], and P. Prenzler[1,2], [1]School of Agricultural & Wine Sciences, Charles Sturt University, Australia, [2]Graham Centre for Agricultural Innovation, Australia, [3]NSW Dept. of Primary Industries, Australia, [4]Inst. for Land, Water, & Society, Charles Sturt University, Australia.

Recently interest has been developing on the enhancement of health-beneficial bioactive compounds present in edible oils (Ghazani et al., 2013; SzydlOwska-Czerniak, 2011). Tocopherols, sterols, and carotenoids are classes of bioactive compounds present in crude canola oil that exhibit substantial health-benefits when incorporated into the human diet. Methods to quantify these compounds in food matrices often involve rigorous sample preparation, resulting in considerable time and cost expenditure (Azmir et al., 2013). A robust HPLC method was developed to simultaneously analyse oil samples for tocopherols, carotenoids, free sterols, and esterified sterols. The use of mass spectroscopy offers enhanced selectivity to enable the determination of both individual free and esterified sterols without saponification, which has not yet been reported for lipid matrices. Furthermore, the use of normal phase liquid chromatography allows the analysis of in-tact lipids, greatly reducing the sample preparation to a single oil extraction and dilution procedure. This method was subsequently used to analyse genotype x environment (G x E) interactions on these bioactive compounds. REML analysis of G x E effects found significant correlations between many of the nutrients and is expected to provide useful information to assist in bioactive retention and overall enhancement of oil quality.

Distribution of Glucosinolates in Camelina Seed Fractions. Y.Y. Shim[1,2], D. Yuan[3], P.D. Jadhav[1], J. Shen[1], V. Meda[3], and M.J.T. Reaney[1,2], [1]Dept. of Plant Sciences, University of Saskatchewan, Canada, [2]Prairie Tide Chemicals Inc., Canada, [3]Dept. of Chemical & Biological Engineering, University of Saskatchewan, Canada, [4]Guangdong Saskatchewan Oilseed (GUSTO) Joint Lab., Dept. of Food Science & Engineering, Jinan University, China.

High glucosinolate concentrations were detected in camelina (*Camelina sativa* L. Crantz.) seed fractions using reversed phase high performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS). The ratio of three glucosinolates (9-methylsulfinylnonyl-glucosinolate, 10-methylsulfinyldecyl-glucosinolate, and 11-methylsulfinylundecyl-glucosinolate) were determined by analysis of extracted ion chromatograms. Total glucosinolate quantitation was performed using proton nuclear magnetic

resonance spectrometry using *N*,*N*-dimethylformamide as an internal standard. The concentrations observed were substantially higher than observed by other methods.

Glucosinolate distribution in *C. sativa* seed fractions produced by pressing and dehulling will be reported. Glucosinolates were highest in defatted meal and lowest in seed oil.

ANA 4: Analysis of Olive and Other Value-added Oils

This session is sponsored in part by Eurofins Central Analytical Labs.

Chairs: R.J. Mailer, Australian Oils Research, Australia; and B. Musselman, IonSense, USA

A Novel Analytical and Chemometric Approach to Survey Quality and Origin of Olive Oil. C. Gertz, Maxfry GmbH, Germany.

The quality of olive oil, defined by sensory characteristics and chemical composition, is affected by different factors such as climate, cultivation, degree of ripeness, conditions of harvesting, transportation and storage of olives, processing techniques, and packaging of the oil. Thus, each olive oil has its unique fingerprint. Many researches have studied the relationship between chemical quality criteria and the organoleptic grading on basis of statistical methods, but the main problem of these approaches was uncertainty (diversity and variability) of data and vagueness of class definitions. Additionally, the number of samples was often low (<200) so that, with increasing number of samples, the variances of the dependent variables in each class increases, too. Thus, multivariate statistical methods are less useful because homogeneity of variances is needed for the statistical evaluation using discriminant analyses. The present work suggests a new approach based on the method of k-nearest neighbours (KNN) as a classification method to determine the quality and authenticity of olive oil. More than 2000 samples of olive oils with different sensory quality from different areas in Europe, Australia, America, and Northern Africa were analysed for parameters given in (EEC) No 2568/1991 using validated NIR methods.

A Survey of Extra Virgin Olive Oils to Test for Adulteration: Application of a Newly Developed FT-NIR Spectroscopic Methodology. M.M. Mossoba[1], H. Azizian[2], A.R. Fardin Kia[1], S.R. Karunathilaka[1], J.K. Chung[1], and J.K.G. Kramer[3], [1]US Food & Drug Administration, USA, [2]NIR Technologies, Canada, [3]Guelph Food Research Center, Agriculture & Agri-Food Canada, Canada.

A recently proposed FT-NIR procedure in conjunction with chemometrics (Lipids, 50:705–718, 2015) for the identification of adulterants in extra virgin olive oils (EVOO) was used to survey commercial products labeled EVOO purchased in the United States and Canada. Potentially adulterated oils were flagged by using (1) gravimetrically-based single-adulterant PLS1 calibration models, (2) determining the concentration of five fatty acids (FA), and (3) estimating the so-called FT-NIR Index. The latter is an indication of quality and an estimate of the volatile components with carbonyl functional group absorbing at 5280cm-1. Based on this FT-NIR protocol, our results were consistent with those of a survey by UC Davis Olive Center published in 2011. Some commercial products were found to be adulterated with refined olive oil and possibly additional amounts of other oils. Others contained one or perhaps more commodity oils other than olive oil. To tentatively verify the presence of multi-adulterants in a few products, selected gravimetric mixtures of EVOO and suspected components were prepared and analyzed. The majority of the EVOO shown to be adulterated by using this FT-NIR methodology could not have been identified based on an altered FA profile. The development of product-specific multi-adulterant calibration models is planned.

Elucidation of Off-flavors in Native Cold-pressed Rapeseed Oils Using the Molecular Sensory Science Concept. M. Granvogl and K. Matheis, Technical University of Munich, Germany.

Rapeseed oil evolved to a major nutrition oil due to its positive physiological properties. Otherwise, flavor attributes are very important criteria for consumers to buy a food. But, up to now, no systematic studies on odorants of rapeseed oil are available.

The lecture will present the characterization of key aroma compounds in native cold-pressed rapeseed oils (both faultless and off-flavor) using the Molecular Sensory Science Concept.

Forty-four odorants were identified in rapeseed oil for the first time, 20 thereof were quantitated via stable isotope dilution analysis, revealing 14 compounds with odor activity values (ratio of concn./odor threshold)= 1 (e.g., 2-isopropyl-3-methoxypyrazine = 330 and dimethyl sulfide = 37).

Comparison of these data with results obtained for the off-flavor oil revealed mainly quantitative and minor qualitative differences, e.g., the concns. of 2-isopropyl-3-methoxypyrazine and dimethyl sulfide decreased, whereas the amounts of 2-isobutyl-3-methoxypyrazine and ethyl 2-methylbutanoate increased. Reconstitution models for both faultless and off-flavor rapeseed oils showed aroma profiles similar to the original oils proving successful identification and quantitation.

With these data at hand and in combination with the knowledge of formation pathways of the respective key odorants, strategies to avoid off-flavor formation can be offered.

Fatty Acid and Sterol Profiles of US-produced Olive Oils. S.C. Wang, Olive Center, University of California, Davis, USA.

In analyzing 194 California olive oils from 2009 to 2013, the UC Davis Olive Center found that 26 percent did not meet the olive oil standards established by the United States Department of Agriculture (USDA) for several fatty acids and sterols. Even though the oils were of extra virgin quality in every other respect, these olive oils would not be considered olive oil under the standard and would be considered in trade as low-value vegetable oil. Since fatty acids and sterols can be affected by varietal, soil, season, and climate, it is of high

importance to collect additional data on these parameters to evaluate the effectiveness of USDA and international olive oil standards. We will discuss the most recent research data on US-produced oils from 2013 to 2016 harvests.

Identification of Olive Oil Cultivars Using LC-MS and Statistical Analysis. B. Seward, C. Stacey, S. Palmer, and R.J. Packer*, PerkinElmer, USA.

Oils of known cultivars from designated areas may be certified for authenticity, and are marketed internationally, commanding a price premium. Different varieties of olives produce oils with particular flavors and stability. This study used LC-MS analysis to determine both the triacylglycerol and polar composition of a large number of Spanish olive oils. Results were analyzed by statistical methods to find distinctive marker compounds. Data fusion methods were explored to combine results from separate analyses for an improved discrimination of cultivars.

GCMS for QC of Plant Essential Oils: Speciation, Authentication, and Stereochemical Determination without Derivatization or Chiral Column. M.W. Bernart and J.J. Plant, Pharmatech Inc., USA.

Plant-derived Essential Oils (EOs) are popular with consumers, partly due to their antimicrobial activities and pleasant aroma characteristics. EOs are complex mixtures of molecules. Gas chromatography (GC) has been the standard analytical method for EOs, due to their complexity and volatility, exemplified by GC methods from International Organization for Standardization (ISO) and Association Française de Normalisation (AFNOR). Thousands of chemical entities, mostly terpenoids, are found in EOs. Hence, many compounds may not be separable during a chromatographic run of reasonable duration. Furthermore, it is cost-prohibitive to purchase the thousands of chemicals needed to build a library of chromatographic retention time standards. Mass spectrometry (MS) coupled to GC separation gives an additional dimension to EO analysis. The mass spectrum of a chromatographic peak is a histogram of % relative abundances of discrete integer masses of molecular ions and their fragments. Spectra of individual compounds have been deposited in digital libraries, where unknowns are screened for spectral matching. We have developed over 60 GC-MS methods for EO analysis, utilizing over 80 different EO standards. The differential response of the mass spectrometer to endo-exo isomerism of bornyl acetates from spruce and hemlock conifer tree EOs will be discussed.

Rice Bran Oil: Challenge of Quality Assessment.
T.H. Tran[1], D.P. Nguyen[1], T.M.T. Nguyen[2], and T. Dao[3], [1]Cai Lan Oils & Fats Industries Co., Ltd., Vietnam, [2]Hanoi University of Science & Technology, Vietnam, [3]HCMC University of Food Industry, Vietnam.

In recent years, Rice Bran Oil (RBO), extracted from rice seed's bran, has been seen as premium edible oil in the world with precious nutrition values. However, oil industry as well as authorities is facing a lot of difficulty in quality assessment of this oil especially in term of acid value, trans fatty acid (TFA). Moreover, foaming issue during frying and color of RBO are subject of many contradictory statements of consumer and oil trader. This study is to provide a detailed evaluation of RBO quality compare to other vegetable oils (soybean oil, palm olein, canola oil) using different standard method (AOCS Ca 5a-40, AOCS Ce 1f-96,…). Mechanism of phenomenon has been explained referring on analysis results of independent institutions. The results showed that each standard method gives very different analysis results. AOCS Ca 5a-40 cannot be applied for testing acid value of RBO due to its special characteristic (high γ-oryzanol). It lacks of evidence to set up suitable method for testing TFA of RBO. Foaming issue is possibly more related to rice specie than processing. These finding can be used as guideline for oil manufacturer and reference for authorities in RBO standardization and commercialization.

Quality Assessment of Extra Virgin Olive Oil with FT-NIR Spectroscopy. D. Behmer[1], C. Gertz[2], and D.E. Roberts*[3], [1]Bruker Optik GmbH, Germany, [2]Maxfry GmbH, Germany, [3]Bruker Optics Inc., USA.

The complex manufacturing process, as well as the growing demand, for extra virgin olive oil results in a high market price, thus making it prone for adulteration. The international olive oil council (IOC) describes various physicochemical parameters and organoleptic methods and standards to define different olive oil qualities. Traditional wet chemical methods become, however, increasingly unacceptable by the industry since they require chemicals and solvents, creating health and safety risks, as well as environmental issues.

To evaluate FT-NIR spectroscopy as an alternative analysis method, more than 300 samples of extra virgin olive oils from different countries, at different age level and qualities were checked by an officially assigned sensory panel. Simultaneously, analytical parameters relevant for the quality, such as fatty acid composition, Anisidine value, free fatty acid content, K-Values Pyropheophytine ,and 1,2-Diglyceride ratio, have been determined.

High calibration accuracy was obtained for the NIR determination of the listed analytical parameters. The results suggest that FT-NIR may offer a powerful tool for the quality control of extra virgin olive oils. Moreover, recent studies show that adulteration with low priced plant oils like hazelnut oil can be detected down to a low percentage range.

Different Vitamin-E Composition and Antioxidant Capacity of *Moringa oleifera* Seed Oil When Extracted by Screw Press versus n-hexane Maceration.
P. Thamakorn and P. Chatthai, King Mongkut's Inst. of Technology, Thailand.

Moringa oleifera seed oil was extracted by either

commercial screw press or by n-hexane maceration for 12h at room temperature, then its vitamin-E composition, antioxidant capacity, and oil stability was determined. All four forms of tocopherols –a, b, g, and d– were found in screw-pressed oil but b-form was absent in the solvent extracted oil, whereas only a-tocotrienol was found in both types of oils. Noticeably, the total amount of vitamin-E in the screw-pressed oil was 1.3 times higher than that in the solvent extracted one. Paradoxically, the antioxidant activity of the solvent extracted oil, determined by DPPH free radical scavenging and FRAP methods, was substantially higher than of the screw-pressed one, probably due to other anti-oxidative substances coming out with the solvent. In agreement with the antioxidant activity, the stability of the solvent-extracted oil, as determined by Rancimat method, was higher than that of the screw-pressed oil.

ANA 5: Advanced Separation Techniques

Chairs: P. Delmonte, US Food & Drug Administration, USA; and G. Purcaro, Chromaleont s.r.l., Italy

Ionic Liquid Capillary Columns for Analysis of FAME Isomers. L.M. Sidisky, G.A. Baney, J.L. Desorcie, and G. Serrano, MilliporeSigma, USA.

Analyses of fatty acid methyl esters (FAMEs) are continuing to gain importance as more research is focusing on their biomedical impacts. This includes the analysis of saturated and polyunsaturated FAMEs, along with the positional geometric FAME isomers. Traditionally, FAME analyses have been performed using polysiloxane or polyethylene glycol phases that yield typical elution patterns. Analysts performing the task of analyzing the fatty acid composition of food have a wide variety of capillary column selectivity's available for resolving the fatty acids as FAMEs depending upon the information they require from their analyses. Nonpolar columns provide a boiling point separation of the isomers with limited resolution of polyunsaturated isomers. Polyethylene glycol columns resolve the isomers by degree of unsaturation with minimal overlap of the carbon chain lengths. The highly polar cyano columns will resolve cis and trans isomers along with possibly providing positional geometric isomer separations depending upon the column type.

New classes of stationary phases based on Ionic Liquid technology have been developed and have demonstrated to provide unique elution patterns for FAME isomers. The two new phases are SLB-IL60 with a PEG like selectivity and the SLB-IL111 with highly polar selectivity. We will compare and contrast the selectivity of the ionic liquid phases with polymeric based phases for various FAME samples.

Multidimensional Techniques for Lipid Analysis. G. Purcaro, Chromaleont s.r.l., Italy.

Multidimensional techniques, such as liquid chromatography hyphenated to gas chromatography (LC-GC) and comprehensive two-dimensional GC (GC×GC), are among the most exciting innovations in gas chromatography since their introduction about 30 years ago. Nowadays, GC×GC can be considered a mature technique to be adopted as a routine control technique in the food quality assessment process. The advantages mostly rely on the possibility to contemporarily perform detailed and sensitive targeted and un-targeted profiling of samples. The have been proven to be very useful both in quality and safety assessment of food, with particular emphasis on the lipid fraction. Both detailed and sensitive targeted and un-targeted analysis can be performed, even simultaneously if a dual detection is employed. The 2D pattern can be handle as fingerprints for samples discrimination and classification.

FAMEs Analysis by Gas Chromatography Vacuum Ultraviolet (GC-VUV) Detection. J.P. Smuts[1], H. Fan[2], L. Bai[2], P. Walsh[1], D.W. Armstrong[2], and K.A. Schug[2], [1]VUV Analytics, Inc., USA, [2]University of Texas in Arlington, USA.

A new vacuum ultraviolet (VUV) detector for gas chromatography was recently developed and applied to fatty acid methyl ester (FAME) analysis. VUV detection features full wavelength spectral acquisition (125–240nm). VUV absorption spectra of several FAMEs types were recorded. Unsaturated FAMEs show significantly different gas phase absorption profiles than saturated ones, and are easily distinguished. Furthermore, VUV allows for the important cis/trans isomer distinction. This is exemplified in the deconvolution of a mixture of cis and trans C18:1 fatty esters closely eluting on SLB-IL 111. As a universal detector, VUV also provides high specificity, sensitivity, and a fast data acquisition rate, making it a powerful tool for fatty acid screening when combined with GC. Several food oil samples are analyzed and demonstrated.

Comprehensive Determination of Saturated Wax Esters in Sunflower Oil Using Ag-ion SPE/GC/FID. L.M. Clement, S.L. Hansen, M. Dao, and R. Fraser, Cargill Inc., USA.

All methods that allow for characterization of wax esters are tedious to some extent. Methods currently used in the industry, including AOCS official method Ch 8-02, were evaluated for wax ester scope, turnaround time, sample size, solvents, consumables, and capital requirements. A new method is introduced that uses silver (Ag) ion solid phase extraction (SPE) with subsequent gas chromatography with flame ionization detection (GC/FID) for the quantitation of saturated wax esters in crude and refined vegetable oils. Theoretical correction factors (TCF) are used for wax ester quantitation relative to heptadecanyl stearate internal standard. This approach utilizes fewer resources compared to other methods, while also providing a more comprehensive profiling of wax esters up to C64. Method validation included accuracy determined by spike recoveries, repeatability determined by triplicate analysis on multiple days using multiple samples, and reproducibility determined by replicate analysis of a common sample by multiple analysts.

Characterization of Alkyl Lipids in Oil Extracted from Sea Cucumber Viscera. J.C. Sullivan Ritter[1], S.M. Budge[2], and R.R. Abuzaytoun*[2], [1]Nature's Way, Canada, [2]Dalhousie University, Canada.

Oil isolated from sea cucumber viscera is gaining attention as an omega-3 rich dietary supplement. It is typically extracted using chemical extraction and contains two unusual classes of lipids. These unusual lipids contain both ether andester bonds and are known as ether or alkyl lipids. Diacylglyceryl ethers (DAGE) contain two ester bonds

and one ether bond while monoacylglyceryl ethers (MAGE) contain one ester and one ether bond. These lipid structures are well-known as essential components of membranes and they serve as antioxidants, protecting the membranes against oxidative stress. In this study, GCMS was used to determine the chain length, branch positions, and numbers of double bonds in the acyl and alkyl portions of these two groups of unusual lipids.

Quantification of Hydroxy Fatty Acids in Fermenting Yeasts with Negative Chemical Ionization Mass Spectrometry: Outcomes, Tips, and Tricks. G. Potter, W. Xia, and S.M. Budge, Dalhousie University, Canada.

The presence of medium-chained hydroxy fatty acids in fermenting yeasts have been known for some time. However, due to the low levels of these free fatty acids, past work could only detect them qualitatively. The danger and poor availability of diazomethane has also required new techniques which still selectively target the free fatty acid fraction but do not use this reagent. Here, were present a novel approach using heptafluorobutyrate derivatives and GC-NCI-MS that allowed for the first quantitative analysis of a fermenting yeast hydroxy fatty acid. With this technique it was possible to assay the levels of 3-OH 10:0 during growth of the SMA strain of *Saccharomyces pastorianus* in lab-scale fermentations. Levels increased between 12-60 hours fermentation time and ranged from 0.68-4.82ng/mg dry weight. While several literature reports have noted the utility of GC-NCI-MS in the past, none have discussed how to implement it. Thus, this study also addresses a number of practical considerations when conducting GC-NCI-MS analysis.

Application of Two Dimensional Gas Chromatography to the Resolution of Fatty Acid Methyl Esters from Complex Animal and Marine Samples. P. Delmonte[1], X. Belaunzaran[2], J.K.G. Kramer[3], and N. Aldai[2], [1]US Food & Drug Administration, USA, [2]University of the Basque Country, Spain, [3]Retired, Agriculture & Agri-Food Canada, Canada.

Comprehensive two dimensional gas chromatography (GCxGC) is emerging as the most proficient separation technique for the resolution of complex mixtures of fatty acid methyl esters (FAME). In this study we compare the separation of FAMEs from animal and marine sources obtained by applying GCxGC, GCxGC modified by the addition of a capillary hydrogenator between the two separation columns, and mono-dimensional GC with highly polar capillary columns. Results will be presented to illustrate how data from different techniques can be integrated, and in particular, how the results from GCxGC can be used to develop accurate mono-dimensional GC methods. The acidic methanolysis of animal tissue lipid extracts produces dimethyl acetals (DMA) in addition to FAMEs. The FAMEs and DMAs of different chain length and degree of unsaturation often coelute making their identification difficult. The reduction of the DMA functional group operated by the capillary hydrogenator provides the resolution of DMAs from FAMEs on two separate regions of the separation plane.

Quantification of Fatty Acids without Calibration Standards Using GC/FID. A.J. Jones[1] and C. Krumm*[2], [1]Activated Research Co., USA, [2]Dept. of Chemical Engineering & Materials Science, University of Minnesota, USA.

The analysis and quantification of fatty acids is typically accomplished with gas chromatography using flame ionization detectors (GC/FID). The variable response of the FID to molecules of different size and composition necessitates *a priori* knowledge of response factors, which are determined from calibrations with pure molecular standards, or, in some cases, from empirical models that approximate them. Calibrations are cumbersome, time-consuming, and often expensive, and the validity of empirical models on different equipment and experimental setups is tenuous. Here, we present the accurate analysis of C8 through C24 fatty acids using GC/FID without the need for calibration or empirical correction factors. A catalytic microreactor is placed directly before the FID to convert all species to methane prior to their detection leading to equivalent detector response on a per carbon basis. The results are compared with those determined using FID with calibrations and theoretical response factors. The increased speed of analysis afforded by eliminating the need for calibrations while maintaining accuracy should lead to increase sample throughput and cost savings for analytical labs.

ANA-P: Analytical Poster Session

Chair: R.A. Della Porta, Frito-Lay, Inc., USA

2. **Fatty Acid Composition and Some Physicochemical Properties of Phoenix Tree Seed Oil.** S. Sun[1], X. Li[1], and Z. Guo[2], [1]Henan University of Technology, China, [2]Aarhus University, Denmark.

Phoenix tree (also called Chinese parasol or Firmiana simplex) is a tall deciduous plant in both sides of road and courtyard in China. However, no available information of Phoenix tree seed oil can be found. In the work, fatty acid composition and some physicochemical properties of the seed oil were investigated.

Results showed that the contents of crude oil, crude protein, moisture, and ash of the seed were 27.8±0.3%, 19.7±0.4%, 7.5±0.2%, and 4.4±0.3%, respectively. The acid value, peroxide value, saponification value, and unsaponifiable matter content of the seed oil were 3.7±0.2mgKOH/g, 2.0±0.2mmol/kg, 183.7±2.4mgKOH/g, and 0.9±0.1g/100g, respectively. The total tocopherol content was 54.5±0.5mg/100g oil (d-tocopherol (29.5±0.6mg/100g oil) and ?-tocopherol (13.8±0.8mg/100g oil)).

GC-MS results showed that the seed oil was rich in linoleic acid (L, 30.2%), oleic acid (O, 22.2%), sterculic acid (S, 23.2%), and palmitic acid (P, 17.4%). The major fatty acids in the sn-2 position of the oil were L (47.9%), O (28.2%) and S (13.4%), and the content (28.1%) of S in the sn-1,3 position was higher than that (13.4%) of sn-2 position. The main triacylglycerols, calculated according to the 1,3-random-2-random hypothesis, were ß-PLS (6.5%), ß-LLS (5.7%), ß-OLS (5.2%), ß-PLL (4.9%), ß-PLO (4.4%), ß-OLL (3.9%), ß-POS (3.8%), ß-SLS (3.8%), ß-LOS (3.4%), and ß-OOS (3.1%), respectively.

3. **Separation of Fatty Acids by SFC.** J. Yang, K.J. Rosnack, J.P. Romano, and P. Alden*, Waters Corp., USA.

Routine simultaneous identification and quantification of a variety of fatty acids is a common analysis in food testing labs. Although gas chromatography (GC) methods are traditionally used for fatty acid analyses (ref 1), they often have long runtime (about 60 minutes), peak shifting issue, and need derivatization. In order to overcome some of these shortcomings, UltraPerformance Convergence Chromatography™ (UPC²) has been investigated for fast analysis of free fatty acids. UPC² is a next-generation supercritical fluid chromatography. It leverages the unique properties of compressed CO_2 at or near its supercritical state, such as low viscosity and high diffusivity, and sub-two micron particle packed columns to improve separation efficiency, speed, and selectivity.

This study will look into the separation of the critical pairs in common fatty acids by UPC². The critical pairs are isomers that are either differ in the trans and cis configuration, such as C16:1(9Z) and C16:1(9E), or the double bond position along the fatty acid chain, such as C18:1(?6), C18:1(?9), C18:1(?11), or combination of both, such as C18:1(6Z) and C18:1(9E). Various UPC² separation conditions, including mobile phase co-solvent and column stationary phase will be investigated.

Reference: 1) Determination of labeled fatty acids content in milk products and infant formula, AOAC Official Method 2012.13 First Action 2012, AOAC International.

4. **Biochemical Characterization and Comparison of Turkish Extra Virgin Olive Oils from Different Olive Varieties of Two Identical Growing Conditions.** C. Dag[1,2], S. Bekiroglu[1], I. Ozdemir[1], O. Sucsoran Karaoglu[1], I. Demirtas[1], M.T. Ozkaya[3], C. Turkay[4], and E. Ertas[1], [1]Food Inst., TUBITAK Marmara Research Center, Turkey, [2]Dept. of Chemistry, Mugla Sitki Kocman University, Turkey, [3]Dept. of Horticulture, Ankara University, Turkey, [4]Alta Horticultural Research Inst. Erdemli, Turkey.

The aim of this study is to determine both the effect of the olive varieties on biochemical characterization of monocultivar EVOO from the same orchard and the effect of the climate and regional variances to the total variance of chemical composition of the EVOOs which produced from the same olive variety. Concerning the same growing conditions on one orchard, the results are used to limit the contribution of parameters such as climate, soil quality, and agricultural practices to the total variance of chemical composition of EVOOs. For this study, we collected 13 different olive varieties from an orchard in Mersin, located at the Mediterranean Sea side of Southern Anatolia. In order to determine the effect of two different origins, i.e. orchards in two different locations, another orchard was selected and the same olive varieties were acquired. The location of the second orchard was in Edremit, which is located at the Aegean region of Western Anatolia, Turkey.

Fatty acid, sterol, and tocopherol compositions were analyzed and the results were compared by multivariate statistical analysis. Principal component analysis (PCA) on the compositional data showed that cultivars can be clearly distinguished on the basis of fatty acid and sterol composition. However, tocopherol composition was relatively less effective in discriminating olive varieties.

5. **Discrimination of Major Cooking Oils in Korea Using Fatty Acid Composition Data in Combination with Canonical Discriminant Analysis.** J. Kim[1], J. Kim[1], H.S. Chun[1], and B.H. Kim[2], [1]Chung-Ang University, Republic of Korea, [2]Sookmyung Women's University, Republic of Korea.

The aims of this study were to investigate the fatty acid composition of the seven different kinds of major cooking oils in Korea and to discriminate between the oils using analytical data in combination with canonical discriminant analysis (CDA). The analytical data were obtained from 35 sesame, 25

sesame flavored, five soybean, five corn, five perilla, five canola, and five sunflower oil samples that were distributed in Korea during 2013 and 2014. Eight variables selected for the CDA were the contents of 16:0, 18:0, 18:1*n*-9, 18:1*n*-7, 18:3*n*-3, 18:1*t*, 18:2*t*, and 18:3*t*. Six different canonical discriminant functions were generated by applying CDA. Four clusters were found in the score plot of the first two canonical functions: Cluster 1 consisted of sesame and sunflower oils; Cluster 2 included sesame flavored, soybean, and corn oils; Cluster 3 was comprised of perilla oil only; and Cluster 4 contained canola oil only. The CDA results showed that all the oil samples were correctly classified. Thus, a combination of fatty acid composition data and CDA is a possible approach to discriminate between the cooking oils distributed in Korea.

7. **A Novel Validated Method for Quantifying Omega-3 Polyunsaturated Fatty Acids (PUFA) in Chewable Gummy Dietary Supplements.** Z. Li[1], E.A. Haile[1], C.J. Oles[2], and C.T. Srigley*[2], [1]Joint Inst. for Food Safety & Applied Nutrition, University of Maryland, USA, [2]Center for Food Safety & Applied Nutrition, US Food & Drug Administration, USA.

Dietary supplements containing omega-3 polyunsaturated fatty acids (PUFA) are widely consumed in the United States (US) to support health and reduce the risk of chronic disease, especially coronary heart disease. Chewable gummy supplements, which are formulated to contain omega-3 PUFA from marine oils, are perceived as more palatable alternatives to conventional soft gels or liquids. However, despite the increasing popularity of these products, a validated method for analyzing their contents of omega-3 PUFA has yet to be reported. The objective of the present study was to develop and validate a method for quantifying omega-3 PUFA in chewable gummy supplements. This procedure involves the hot acidic dissolution of homogenized gummy samples followed by liquid-liquid extraction with low toxicity solvents. Extracted lipids are then converted to fatty acid methyl esters according to AOCS Official Method Ce 2-66 and analyzed by gas chromatography with flame ionization detection. This method shows excellent performance in method validation studies, including repeatability/precision, accuracy, robustness, and absence of interferences or contaminant carryover. Overall, the current method is appropriate for quantification of omega-3 PUFA, and other fatty acids, in the wide variety of chewable gummy dietary supplements available in the US market.

8. **Optimization of Methylation Conditions for the Quantification of a-Eleostearic Acid in *Njangsa* (*Ricinodendron heudelotii*) Seed Oil.** H.K. Abaidoo-Ayin[1], P.G. Boakye*[1], K.C. Jones[2], V.T. Wyatt[2], and S.E. Lumor[1], [1]Delaware State University, USA, [2]USDA, ARS, ERRC, USA.

Interest in conjugated fatty acids (CFAs) has grown in recent years due to its associated health benefits. Currently, seed oils remain the major natural source of CFAs. Studies have shown that one such seed oil, *Njangsa* (*Ricinodendron heudelotii*), contains significant amounts of a-eleostearic acid (a-ESA), a conjugated isomer of linolenic acid. However, the exact amount of a-ESA in the oil has not been firmly established. Some authors have suggested that a-ESA easily undergoes isomerization, leading to questionable results. Our hypothesis is that the conditions used for the preparation of fatty acid methyl esters (FAME) prior to instrumental analysis affects the stability of a-ESA and therefore impacts the total yield of the CFA. To achieve our aim, we used a 2x5x7 factorial design with factors including type of catalyst (acid, base), temperature (30–70°C), and incubation time (5–120 minutes). Our initial findings show that for similar temperature and time combinations, base-catalyzed methylation yielded higher levels and less isomerization of a-ESA acid than acid-catalyzed methylation. We are currently monitoring the effect of increasing temperature and time in order to obtain the optimal conditions for quantification of a-ESA acid in *Njangsa* seed oil.

9. **Characterization of Chicken Yolk Vitelline Membrane Proteins Using Eggs Enriched with Conjugated Linoleic Acid.** S.E. Shinn ***Honored Student and The Peter and Clare Kalustian Award Winner)***, R. Liyanage, A. Proctor, and J.O. Lay, University of Arkansas, USA.

The chicken egg vitelline membrane (VM) separates the yolk from albumen. The objective of this study was to determine protein differences in VM of conjugated linoleic acid (CLA) rich eggs, relative to control egg VM. Recently, we characterized the lipids of VM using direct MALDI TOF/MS, without any prior chemical derivatization. We determined that CLA eggs possessed VM lipids with significantly different abundancies of 9 phosphatidylcholine species and 6 triacylglycerol species. Other significant differences appeared after 20 days of refrigerated storage. This study used the same rapid, direct MALDI technique to compare protein mass spectra of egg yolk VM in CLA-rich, soy control, and standard control eggs, at day 0 and day 20. Fresh VM from CLA-rich yolks had reversed ratios of gallin protein precursors (m/z 4484 and 4597) on the inner membrane layer, relative to both controls. After 20d of refrigerated storage, gallin protein ratios were similar among treatment groups, but standard control VM contained significantly greater levels of gallin proteins on the inner membrane layer, and significantly lower gallin protein levels on the outer membrane layer. This rapid MALDI analysis allowed collection of lipid and protein mass spectra by simply shifting the mass range during analysis.

11. **Double Bond Distribution Structure of Triacylglycerols in Vegetable Oils.** B. Xue and W. Cao*, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., China.

Tandem silver ion HPLC columns, polybasic solvents blending gradient elution and evaporative light scattering detection was applied to establish a silver ion HPLC method,

which can analyze 18 different standard types of triacylglycerol(with 0 to 9 double bonds) based on triacylglycerol's double bond difference in both amount and distribution structure within 50 minutes. Double bonds distribution type(molecular composition) of 18 standard triacylglycerols and elution order was as follow:000(SSS)>010(SOS)>001(SSO)>>020(SLS)>011(SOO)>101(OSO)>>111(OOO)>012(SOL)>>022(OLL)>202(LOL)>121(OLO)>112(OOL)>>122(OLL)>212(LOL)>>113(OOLn)>>222(LLL)>>223(LLLn)>>333(LnLnLn). Then, complex triacylglycerols from 11 kinds of vegetable oils was analyzed, which included soybean oil, maize oil, rapeseed oil, rice bran oil, sesame oil, peanut oil, sunflower oil, oil-tea camellia seed oil, olive oil, flaxseed oil, and palm oil in China. The result was found that, except for olive oil and oil-tea camellia seed oil, there was distinct difference between all kinds of vegetable oils in double bond distribution structure of triacylglycerols, and that many types of triacylglycerol in vegetable oils was out of 18 types of standard triacylglycerol.

12. **Asarinin as a Specific Indicator for Identification of Roasted Sesame Seed Oil Adulterated with Refined Unroasted Sesame Seed Oil.** W. Cao[1], C. Yuan[2], B. Xue[1], and F. Chen[2], [1]Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., China, [2]Shanghai Grain Science Research Inst., China.

Two different types of sesame oil, roasted seed oil (RSO) and refined unroasted seed oil (RUSO), are produced in Asia. Some of the firms are trying to maximize profits by adulterating the RSO with the RUSO. The objective of this study was to find a new effective identification method. The difference in the composition of sesame lignans between the RSO and the RUSO was analyzed by the Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Results showed that the RSO contained a considerable amount of sesamin and sesamolin, and the RUSO contained an unknown substance except for sesamin and sesamolin. The unknown substance was separated from the RUSO by Preparative Flash Chromatography and was further purified by Preparative High Performance Liquid. After analyzed by NMR and MS, the unknown substance was identified as asarinin, an isomer of sesamin. The study also indicated that in the commercial preparation of sesamin epimerization occurred during acid-clay bleaching in an oil refining process and a mixture of sesamin and asarinin in almost equal amounts was formed. The study showed that asarinin was a specific indicator for identification of the RSO with the RUSO.

13. **Green Method for Authentication of Lipid Saturation/ Unsaturation Using Natural Frankincense Resin.** A. Hayyan[1,2], M.E.S. Mirghani[3], S.N. Rashid[1,2], M. Hayyan[2,4], and M.A. Hashim[1,2], [1]Dept. of Chemical Engineering, University of Malaya, Malaysia, [2]University of Malaya Centre for Ionic Liquids (UMCiL), Malaysia, [3]Dept. of Biotechnology Engineering, International Islamic University Malaysia, Malaysia, [4]Dept. of Civil Engineering, University of Malaya, Malaysia.

Frankincense resin (Olibanum) is natural plant resin and an edible ingredient material. Due to aromatic components in its chemical this material was used widely in Arabic Gulf countries as an incense. This study used frankincense resin as an additive material to investigate the saturation/unsaturation level of vegetable oils such as olive oil and palm oil. The methodology was based on the direct solubility of frankincense resin in oils and the sample of frankincense were obtained from Sultanate of Oman. FTIR spectroscopy was used to detect chemical function groups. The results showed that the solubility of frankincense was varied based on saturation/unsaturation level of oils. The functional group at 680–660cm^{-1} assigned for alkyne C–H bend, appears as a result of frankincense treatment and the functional group (C–O stretch vibration) was appeared clearly at wave length of 1033 and 1063 cm^{-1} in olive oil treated with frankincense while these bands were not appear in palm oil after treated with frankincense. This is the first study to use frankincense as natural material to check the source of lipids. This method is considered as a new support method for FTIR and E-nose techniques for detection oil source and its saturation level. This green method can open the door for application of aromatics materials to be used to study chemistry of lipids and their analysis.

14. **Qualification of Gangliosides in Milk Products by NMR Spectroscopy.** B.W.K. Diehl and S. Lützenkirchen, Spectral Service AG, Germany.

Phospholipids can be derived from several natural sources. Besides soybeans and egg yolk, milk contains several phospholipids (PLs) and glycolipids. A sub-category of glycolipids, only existing in sources of animal origin, is gangliosides. Gangliosides themselves possess several beneficial properties, especially for human infants. A nuclear magnetic resonance (NMR) spectroscopic method was developed to analyze rapidly gangliosides in milk products.

In ^{1}H NMR spectra of milk powder characteristic signals of gangliosides were detected, generated by the ganglioside specific sugar molecule N-Acetylneuraminic acid (Neu5Ac). Number of signals depends on the number of Neu5Ac in one ganglioside. GD3, the predominant ganglioside in milk, shows an additional methylene signal in contrast to GM3 with the second largest quantity in milk. Overlapped signals could be identified by 2D-H,H-COSY-NMR. The gangliosides GM3 and GD3 could be differentiate by the different number of signals triggered by NH group of Neu5Ac. The well separated signals could be used for quantification of gangliosides (RSD < 5.6%).

Ganglioside specific signals were identified in ^{1}H and 2D-H,H-COSY spectra. In milk powder samples the amine group was suitable for fast ganglioside qualification. Due to fast and reliable measurement the NMR spectroscopy is an excellent opportunity for existing analytical methods in ganglioside

analysis.

15. **Characterization and Quantification of Glycolipids from Vegetable Lecithin by NMR Spectroscopy.** B.W.K. Diehl and S. Jensch, Spectral Service AG, Germany.

Vegetable lecithins are the main source of commercially used phospholipids (PLs), containing also glycolipids. Glycolipids show similar properties like PLs, being used as surfactants in pharmaceutical and cosmetic fields as well as in the food industry, where they are known for their excellent baking potential. A nuclear magnetic resonance (NMR) spectroscopic method was developed to analyze and quantify glycolipids from vegetable lecithins without the separation from phospholipids within short time.

Four different glycolipids were identified: Monogalacotsyl-digliceride (MGDG), Digalactosyl-digliceride (DGDG), Sterylglycoside (SG), and Acyated-Sterylglycoside (ASG). Signals related to olefinic protons of the sphingosine backbone were found in the ^{1}H NMR spectrum in low intensities. Quantification of the four glycolipids by characteristic signals was possible from the ^{1}H NMR spectrum of raw lecithin (MGDG, DGDG) and de-oiled lecithin (SG/ASG). The ratio of SG/ASG could be obtained from ^{13}C or 2D NMR spectra as well as the differentiation between Sitosteryl- and Stigmasterylglycoside.

Quantification of the main glycolipids from vegetable lecithin is possible by ^{1}H NMR. The prepared sample was found to be suitable for the qualitative and quantitative measurement of phospholipids by ^{31}P NMR and glycolipids by ^{1}H NMR.

16. **^{1}H NMR Inter-laboratory Test for Edible Oil Characterization.** B.W.K. Diehl, E. Zailer, and Y.B. Monakhova, Spectral Service AG, Germany.

The NMR spectroscopy is a technique which is accurate and precise enough to rival chromatographic and titration methods, especially in the edible oils analysis. The successful participation in inter-laboratory tests is a suitable opportunity to ensure the ability of NMR laboratories to provide accurate and precise results. In order to become accepted as a valid method by public authorities, intra- and inter-laboratory tests are organized for instance to verify the reproducibility of NMR spectroscopy and to check the operator-to-operator and instrument-to-instrument variability on the content values.

An intra-laboratory test is performed to show the influence of sample preparation and integration procedure on the accuracy and precision of NMR spectroscopy. Six laboratory technicians prepared five different edible oils by weighing and solving the sample. The integration is carried out semi-automatically.

Fifteen national and international laboratories prepared five different, independent edible oil sample solutions which were measured with the same method by different NMR equipment (e.g. difference in magnetic field strength and type of (cryo) probe). The NMR spectra were integrated by professional qualified evaluators.

18. **Application of Gas Chromatography–vacuum Ultraviolet Absorption Detection for the Analysis of Fatty Acid Methyl Esters.** C.A. Simpson[1], H. Fan[2], J.P. Smuts*[2], L. Bai[1], P. Walsh[1], D.W. Armstrong[2], and K.A. Schug[2], [1]VUV Analytics, Inc., USA, [2]Dept. of Chemistry & Biochemistry, University of Texas at Arlington, USA.

Fatty acids and their corresponding methyl esters (FAMEs) are important analytes for consideration in terms of food science, nutrition, and bio-based fuels. Typically, these are characterized by gas chromatography–mass spectrometry (GC-MS), but the complexity of the system, as well as many closely related isomers and isobars, can make complete speciation difficult. We have applied a new vacuum ultraviolet absorption detector for GC (GC-VUV) to demonstrate its superior capability for FAME characterization. GC-VUV measures the absorption of eluting compounds in the 115–240nm range where all chemical species absorb. Each FAME and class of FAME have unique absorption features that enable both qualitative and quantitative analysis. The differentiation of FAMEs is demonstrated with standard mixtures, as well as mixtures of FAMEs prepared from various food oils. GC-VUV is shown to be extremely well adept at characterizing FAME compositions from real oil samples without significant interferences.

19. **Essential Oil Yield and Characterization of 14 *Ocimum tenuiflorum* Varieties.** N.J. Fuller, R.B. Pegg, and D.C. Berle, University of Georgia, USA.

With rising healthcare costs, there is an increased focus on the role of medicinal plants in health and wellness. Holy basil (*Ocimum tenuiflorum*) is an important medicinal used to reduce stress, regulate metabolism, and reduce inflammation. This study evaluated the essential oil content and composition of *O. tenuiflorum* varieties to determine the best for commercial production.

Plants from 14 holy basil varieties were selected from commercial catalogs and the USDA Germplasm systems. Plants were grown in the field, harvested, and biomass recorded before and after drying. Essential oils were extracted by hydrodistillation. Essential oils were analyzed qualitatively by GC-MS and quantitatively by GC-FID. The varieties were ranked according to the essential oil yield per plant.

The top five yielders included both USDA and commercial varieties, including PI288779, Amrita, PI652059, PI652057, and Kapoor. Major compounds identified in the essential oil include eugenol, caryophyllene, ß-bisabolene, methyl eugenol, estragole, and ß-elemene. Eugenol is one of the most desired compounds in the essential oil, and its content varied significantly amongst varieties.

The findings suggest an inverse relationship between biomass yield and essential oil content, and different

essential oil chemotypes that should all be considered when choosing a variety for commercial production.

20. **Modern State of GC-MS/MS Pesticide Analysis and Workflows for Unstoppable Productivity.** L.A. Dolata, Thermo Fisher Scientific, USA.

Triple quadrupole mass spectrometers systems have gained popularity over their single quadrupole counterparts because of their high selectivity and lower detection limits, especially in complex matrices such as those encountered in pesticide analysis in food. In this poster we present results of GC-MS/MS analysis of pesticides using timed-Selective Reaction Monitoring (t-SRM). The t-SRM optimized dwell times combined with the Enhanced Velocity Optics (EvoCell collision cell) present in modern day triple quadrupole technology enables us to monitor multiple confirming transitions per analyte for a more confident confirmation without compromising quantitation sensitivity. The results we show were obtained using a state-of-the-art CDS software platform, which combines powerful data analysis capability with easy pesticide analysis method creation. The pesticide analyzer database contains retention times and transitions for over 1000 pesticides and other compounds of environmental interest. Historically, developing MS/MS transitions for compounds used to be arduous and time-consuming process prone to operator error, but this no longer is the case. This poster highlights the power of AutoSRM which is a tool for developing and optimizing transitions for compounds that are not yet present in the database with simple user interaction and high degree of confidence in the results.

21. **Elucidation of Minor Components in Vegetable Oils by Using Comprehensive Two-dimensional Gas Chromatography: A "Green" Evolution.** G. Purcaro[1], F.A. Franchina[1], L. Barp[2], P.Q. Tranchida[2], and L. Mondello[1,2], [1]Chromaleont s.r.l., Italy, [2]Dipt. di Scienze Chimiche, Biologiche, Farmaceutiche, ed ambientali, University of Messina, Italy.

The present research is related to the investigation of minor components in vegetable oils, namely fatty acid ethyl esters (FAEEs), sterols, and waxes, exploiting the advantages of a solid phase extraction (SPE) and the potential of comprehensive two-dimensional gas chromatography (GC×GC), coupled to a mass spectrometer (MS) and a flame ionization detector (FID).

Particularly, and starting from the initial EEC 2568/91 Regulation, an "environmentally-friendly" and more effective evolution of the entire method will be shown; the method proposed is essentially a unified one, enabling the simultaneous determination of FAAEs, sterols and waxes. The sample preparation method was first reduced from a chromatographic column separation involving 15g of sorbent and about 220mL of solvent to an SPE-scale purification, using 3g of sorbent and about 50mL of solvent. The pre-separation was further miniaturized using a Pasteur pipette to perform a miniaturized SPE (500mg of sorbent and about 5mL of solvent). The oil sample was previously derivatized to enlarge the number of lipidic class considered in a single analysis.
The final determination was carried out using GC×GC-FID/MS. Both cryogenic and flow modulators were optimized proving the effectiveness of the flow modulator, which represents a cheaper and more environmental-friendly alternative compared to the cryogenic modulator.

22. **Lipidomics-type Analysis of Human Plasma by UHPLC-qMS.** M. Beccaria[1], V. Inferrera[2], F. Rigano[1], P. Dugo[1,2], G. Purcaro*[1], and L. Mondello[1,2], [1]Chromaleont s.r.l., Italy, [2]Dipt. di Scienze Chimiche, Biologiche, Farmaceutiche, ed Ambientali, University of Messina, Italy.

The high structural diversity in lipid classes present in biological samples makes their characterization a very challenging task. However, chromatographic techniques, and in particular liquid chromatography hyphenated to mass spectrometry (LC-MS) provides several advantages over direct infusion techniques (e.g. shotgun MS). The aim of this work was to develop a simple, fast, and versatile chromatographic method to be applied to an ultra-high performance LC (UHPLC) system coupled to a single quadrupole (q)MS. The method developed was suitable for both electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) interface, for untargeted lipid profile characterization of human plasma. Furthermore, the capability of qMS to work in positive- and negative-ion mode simultaneously was exploited. Without any modification of the chromatographic conditions (mobile phase, flow, injection volume, gradient, etc.). Thus, a simple comprehensive platform was provided to in-depth studies in lipidomics.