106th AOCS Annual Meeting and Industry Showcases

Analytical Division Technical Program Abstracts

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ANA 1: Trace Contaminants

Chairs: J.D. Pinkston, Kellogg Co., USA; and K. Hrncirik, Unilever R&D, The Netherlands

Key Achievements and Challenges on the 3-MCPD Issue Journey. K. Hrncirik, Unilever R&D, The Netherlands.

Within the last few years the presence of 3-monochloropropan-1,2-diol (3-MCPD) esters and glycidyl esters in refined vegetable oils received significant attention of chemists, food technologists, toxicologists and other professionals. Since refined oils are used in a wide range of food products (either as an ingredient or as a medium during the food preparation), there is a great interest in developing effective mitigation technologies, and thereby reducing the exposure to these contaminants. Along this long-term quest, several challenges and knowledge gaps have emerged, namely the availability of analytical methods for reliable quantification, the elucidation of the mechanism of formation and the absence of the overall risk assessment. This speech provides a review of major milestones achieved over past years and, at the same time, addresses several challenges we face on the way to resolve this issue.

Determination of MCPD Esters and Glycidyl Esters in Processed Food. T. Wenzl, V. Samaras, A. Giri, Z. Zelinkova, L. Karasek, and G. Buttinger, Institute for Reference Materials and Measurements, Belgium.

Information on methods to determine 3-monochloropropanediol (3-MCPD) esters, 2-monochloropropanediol (2-MCPD) esters, and glycidyl esters in processed food is scarce. However, the European Commission asked in 2014 EU Member States to monitor these substances in food and requested the European Food Safety Authority (EFSA) to come up with a scientific opinion. In support to EFSA and the European Commission, the Joint Research Centre developed an analysis method for the simultaneous determination of MCPD esters and glycidyl esters in food. Special attention was given to low LODs, which were for all analytes below 20 µg/kg fat. The analysis method is based on a mild extraction of fat from food followed by the conversion of glycidyl esters into monobromopropanediol (MBPD) esters. A defined portion of the fat is subjected to acidic transesterification and extraction of the free forms of MCPD and MBPD into an organic solvent, in which they are derivatised with phenyl boronic acid (PBA) prior to GC-MS measurement. The analysis method was tested on breads, fine bakery wares, smoked fish, meat products, potato and cereal based snacks, and margarines. The presentation will provide method details, and report on experiences made in the analysis of more than 500 samples.

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Detection of Glycidyl Esters and MCPD Esters in Infant Formula. S. MacMahon, J. Leigh, L. DeJager, and T. Begley, US Food and Drug Administration, USA.

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are potentially carcinogenic contaminants formed during the processing of commonly consumed edible oils. While there are several methods for the analysis of these contaminants in oils, validated methodology in complex foods are currently limited to mayonnaise and dressings. As these oils provide the primary source of fats in infant formulas, validated methodology is needed to determine infant exposure to 3-MCPD, 2-MCPD and glycidyl esters.

The approach described in this presentation has been validated for the detection and quantitation of intact 2-MCPD, 3-MCPD and glycidyl esters in powdered infant formula. The development of conditions to quantitatively extract these contaminants from infant formula will be discussed. The infant formula extract is then cleaned up by solid-phase extraction prior to LC-MS/MS analysis. This direct approach is rugged, sensitive, specific, and allows for the determination of fatty acid esters of 2-MCPD, 3-MCPD, and glycidol in infant formula using methodology suitable for regulatory analysis.

A Rapid Indirect Method for Simultaneous Determinations of 2-/3-MCPD Esters and Glycidyl Esters in Foods. K. Miyazaki and K. Koyama, House Foods Group Inc., Japan.

Fatty acid esters of 2-chloro-1, 3-propanediol (2-MCPD-Es) and 3-chloro-1, 2-propanediol (3-MCPD-Es), and those of glycidol (Gly-Es) reported to occur in refined vegetable oils and fats are causing public health concerns. The rapid indirect method we had developed earlier by using a *Candida rugosa* lipase for simultaneous determinations of 2-/3-MCPD-Es and Gly-Es in edible oils and fats was successfully applied to the analyses of foods containing oils and

fats. When mayonnaise, vegetable oil margarine and fat spreads, spiked with 3-MCPD dioleate, 2-MCPD dilinolate and Glycidyl oleate, each at 20 µg/g as ester, were analyzed, recovery rates for 3-MCPD, 2-MCPD and Gly were 93-108%, 99-106% and 96-105% respectively, even without fat extraction. Although similar recovery rates for 2-MCPD and Gly were obtained from butter and margarine high in milk fat, the recovery rates for 3-MCPD were lower (73-89%). By employing fat extraction with mixture of t-butyl methyl ether and isooctane, the recovery rates for 3-MCPD were improved to 93-103%. Similarly, by increasing the temperature for ester hydrolysis from room temperature to 40°C, they were improved to 92-107%. After the fat extraction, infant formulas yielded recovery rates of 99-107%, 94-104% and 95-101% for 3-MCPD, 2-MCPD and Gly, respectively.

Determination of Brominated Vegetable Oil in Soft Drinks by UPC^2-MS. J. Yang and J. Romano, Waters Corp., USA.

Brominated vegetable oil (BVO) is often used as a weighting agent, or a solubility-transmitter for citrus oils and other lipophilic compounds[1] in soft drinks and beverages. US FDA has established a BVO limit at 15 ppm in finished beverages, while many countries have banned its use in beverages. Analysis of BVO is rarely reported. Gas chromatography with mass spectrometry (GC-MS) has been proposed recently for the analysis of BVO in soft drinks and cocktail syrups[2,3]. This GC-MS method requires tedious derivatization (or saponification) of BVO, and has a long run time (about 50min).

UltraPerformance Convergence Chromatography™ (UPC^2®) is a state-of-art supercritical fluid chromatography (SFC) that provides exceptional efficiency and speed of separation[4]. It has been applied to a wide range of compounds, including VO, and has shown great benefits in selectivity, throughput, and ease-of-use[5]. This work demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC^2-MS. BVO was extracted and analyzed directly without any derivatization. The chromatography total run time was 9 min. The analytical method performance (limit of quantitation or LOQ, repeatability, linearity, and recovery) as well as the analysis of BVO in soft drinks and beverages are presented.

Refining of Vegetable Oils and Fats: Formation Pathway and Mitigation of 3-MCPD and Glycidyl Esters. K. Bhaggan and J. Werleman, IOI Loders Croklaan, The Netherlands.

Oil refining process has been continuously improved to remove contaminants like pesticide, poly-cyclic aromatic hydrocarbons, dioxins and avoid formation of unwanted by-products.

According to the latest scientific knowledge, 3-MCPD esters and glycidyl esters are formed in during the refining process. Especially in refined palm oil high concentration of these esters are detected. The formation pathway is still not known although there have been several hypothesis published regarding the formation of 3-MCPD and glycidyl esters.

Since refined palm oil is used either as ingredients or as medium during the processing in various food applications and infant formula, there is a need to reduce the level of these processing contaminants. Different possible processing routes have been investigated to reduce these contaminants in refined palm oil.

This presentation concentrates on either different ways to prevent the formation of 3-MCPD and glycidyl esters during refining or to reduce the level of these contaminants refined palm oil or it fractions. Different refining steps as well as modification processes are taken into consideration and several processing aids and effect of minor components have been investigated.

Refining of Vegetable Oils: Influence of Refining Parameters on Mitigation of 3-MCPD and Glycidyl Fatty Esters. K. Schurz, Clariant Produkte (Deutschland) GmbH, Germany.

In 2006 3-MCPD fatty acid esters have been detected in fully refined vegetable oils and fats. Predominantly in processed palm oils high values for 3-MCPD fatty esters and even more of the also harmful glycidyl fatty acid esters have been identified with particular relevance to baby food. “Market opinion” saw bleaching earth as a possible contributor.

Target was to clarify the role of bleaching earth, the impact of refining steps prior to bleaching – namely neutralization and degumming – and the possibility to mitigate formation resp. removal of fatty esters of 3-MCPD (hereinafter referred to as 3-MCPD) and glycidol from the fully refined product. The experiments focused on crude palm oil having the most importance.
It could be demonstrated that

- Neutralization of the oil enables low values for 3-MCPD
- Degumming influences remarkably the 3-MCPD values depending on type and dosage of the acid used
- Bleaching earth lowers the potential for the formation of 3-MCPD depending on
    - Type
    - Analytical parameters
    - Dosage
    - Temperature
    - Presence of acids
- Post-treatment of fully refined palm oil or its fractions with bleaching earth reduces glycidyl fatty esters depending on
    - Type of bleaching earth
    - Presence of acids

As a result of the described experiments it is obvious that refining parameters prior to the final deodorization step allow mitigation of 3-MCPD.

Digestion of 2- and 3-MCPD Esters by Pancreas Lipase and Estimation of Their Intestinal Absorbability. N. Kaze[1], H. Sato[2], K. Murota[3], S. Kumamoto[3], M. Kotaniguchi[4], S. Kitamura[4], H. Inui[4], H. Yamamoto[1], and Y. Watanabe*[2], [1]Ueda Oils and Fats MFG, Japan, [2]Osaka Municipal Technical Research Institute, Japan, [3]Kindai University, Japan, [4]Osaka Prefecture University, Japan.

3-monochloropropane diol (MCPD), undesired contaminants in fats and oils exists as an ester form with two molecules of fatty acids. 2-MCPD, the isomer, also exists in foods as an ester form, but its toxicity is not yet confirmed. In order to estimate the profile of 2-MCPD esters after the intake, 2- and 3-MCPD dioleate was hydrolyzed by porcine pancreas lipase and pancreatin. As previously reported, free 2-MCPD was the major hydrolysate of 2-MCPD dioleate. In contrast, 2-oleoyl-3-MCPD was the major one from 3-MCPD dioleate, when determined by HPLC-Corona CAD. The hydrolytic property of the mixture of 1-, and 2-oleoyl-3-MCPD by the lipase was consistent with the previous observation; the amount of the former reduced whereas the latter did not. 2-MCPD penetrated Caco-2 cell monolayer, which is the epithelial cell model, at the similar speed with 3-MCPD. Surprisingly, the rate limiting step was estimated to be the penetration or diffusion through the polymeric film of Transwell, rather than the cell monolayer. The tight junction of the cell monolayer was affected little by the exposure to the compounds. In our preliminary study, the level of MCPDs in the blood increased in several minutes after the dose to the intestine in rats.

ANA 2: Advanced Separation of Lipids/Multidimensional Techniques

Chairs: W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA; and P. Delmonte, US Food and Drug Administration, USA

The Updated Bottom-up Solution: Using Critical Ratios for Triacylglycerol Structural Analysis by Mass Spectrometry. W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA.

Ratios of fragments in mass spectra of triacylglycerols (TAGs) called 'Critical Ratios' provide structural information about TAGs, such as the degree of unsaturation (number of double bonds) and the positions of the fatty acyl chains on the glycerol backbone (regioisomers). Data from an LC1/MS3 'triple parallel mass spectrometry' experiment will be used to describe how critical ratios relate to TAG structure, using data from atmospheric pressure chemical ionization (APCI) mass spectrometry (MS), electrospray ionization (ESI) MS, and product-ion ESI-MS/MS
The ratio of the protonated molecule, $[M+H]^+$, to the diacylglycerol-like fragments, $[DAG]^+$, called the MH/?DAG critical ratio obeys a model that was presented in a previous book chapter. In addition to describing how the critical ratios can provide structural information at face value, the critical ratios represent a "reduced data set" that can be used to reproduce the raw mass spectra from which the ratios came, at any time. However, it requires fewer values in the form of critical ratios than values in the form of ion abundances to completely express the data, thus making the critical ratios a 'reduced data set'. Furthermore, the critical ratios provide more information about the TAGs using fewer values than the raw mass spectra.

Potentiality of the GC×GC Technique for Quality and Authenticity Assessment of Olive Oil. G. Purcaro[2,3], L. Mondello[1,3], and L. Conte[2], [1]University of Messina, Italy, [2]University of Udine, Italy, [3]Chromaleont s.r.l., Italy.

Comprehensive GC (GC×GC) can be considered a mature technique to be adopted in routine controls in the food quality and purity assessment. The advantages mostly rely on the possibility to contemporarily perform detailed and sensitive targeted and un-targeted profiling of samples in a single chromatographic run. Both volatiles and minor components of extra virgin olive oil have been investigated by GC×GC, coupled to dual detectors MS/FID, obtaining a multiple levels of information from each single analysis. The volatile fraction of a series of extra virgin olive oil samples has been characterized. Complex 2D patterns have been adopted for samples' classification. Samples' sensory attributes, and in particular specific aroma defects responsible of samples declassification, defined by an official Panel test, have been combined with the GC×GC aroma blueprint to establish correlations and locate informative markers to be profitably screened in a routine quality assessment.

Minor compounds of vegetable oil were obtained employing a derivatization step prior to a silica column separation reducing manipulation and thus artefacts deriving from saponification procedure. Free sterols and others compounds were eluted in the same fraction of waxes and alkyl esters, thus allowing to explore a "comprehensive" fingerprint of different oils in a single analysis.

Multidimensional Gas Chromatographic Techniques Applied to the Analysis of Lipids from Marine Species of the Mediterranean Sea. R. Costa[1], A. Albergamo[1], M. Oteri[1], M. Piparo[1], G. Purcaro[3], P. Dugo[1,2], and L. Mondello[1,2], [1]University of Messina, Italy, [2]University Campus Bio-Medico of Rome, Italy, [3]Chromaleont s.r.l., Italy.

Sea and its living organisms constitute samples of high interest in environmental analysis. They provide reliable information about: 1) pollution level; ii) nutritional value and toxicology of species entering the human food chain; iii) metabolic pathways of biological interest. In a preliminary stage, three different extraction methodologies were tested, namely Folch's, Bligh & Dyer's and maceration. Furthermore, prior to GC×GC analysis of fatty acid methyl esters (FAMEs), two different transesterification procedures were applied, in order to estimate the effects depending upon the derivatization process. Once optimized the sample preparation steps, FAMEs obtained from mussels (*Mytilus galloprovincialis*) and clams (*Venerupis aurea* var. *laeta*) autoctonous of Ganzirri lake (neighbourhood of Messina, Sicily); and from muscle tissues obtained from fish of the species *Sparus aurata* and *Dicenthrarcus labrax,* were analyzed by comprehensive GC. The high separation power of the two-dimensional approach along with the group-type pattern formation over the 2D space of the various fatty acid families, allowed to obtain detailed compositions of the samples investigated. The identification process was also supported by mass

spectrometric detection, with dedicated mass spectral database.

Monodimensional Gas Chromatographic Separation of Fatty Acid Methyl Esters from a Two Dimensional Perspective. P. Delmonte, US Food and Drug Administration, USA.

The development of more polar stationary phases for gas chromatography allowed for improved separations of fatty acid methyl esters (FAMEs) based on the number and geometric configuration of double bonds, but accompanied by an increased overlap of FAMEs with different number of carbons and double bonds. Comprehensive two-dimensional gas chromatography (GC×GC) was successfully employed to detect these coelutions, to quantify the unseparated FAMEs, and to optimize the elution parameters in order to minimize overlaps. The elution and quantification of FAMEs prepared from commercial fats and oils obtained with highly or extremely polar capillary columns were evaluated by GC×GC, using the second dimension capillary column to resolve the FAMEs with different chain lengths not separated by the first one. This presentation will dedicate particular attention to the separation and quantification of the polyunsaturated *trans* fatty acids contained in refined non-hydrogenated oils.

LC-GC-FID Technique as a Powerful Tool for Olive Oil Analysis. T. Küchler[1], H. Boysen[1], M. Nestola[2], and P. Tablack[2], [1]Eurofins Analytik GmbH, Germany, [2]Axel Semrau GmbH & Co. KG, Germany.

For the judgment of the quality and purity of extra virgin olive oil (EVOO) several methods are described in the scientific literature resp. are part of obligatory regulations, e.g. the rules of the International Olive Council (IOC). Most of these methods require a high effort for the sample preparation. With the online-coupled LC-GC technique many manual sample preparation steps can be avoided. Two important methods for the analysis of EVOO are presented: One new quality parameter is the content of fatty acid ethyl esters, which is a direct marker for the quality of the olives that were used for the production of the EVOO. Another parameter is the content of sterols as well as the profile of the different sterol isomers which are directly connected with the purity of the EVOO. For both parameters, LC-GC-FID methods were developed and validated as well as compared with the recent standard methods. It was shown that the LC-GC-FID is a good alternative technique which delivers comparable results with the standard methods and saves a big part of time and effort.

Evaluation of Fully Comprehensive and Selective Comprehensive Two-dimensional Liquid Chromatography for the High Resolution Separation of Triacylglycerols. D. Stoll[1] and P. Delmonte[2], [1]Gustavus Adolphus College, USA, [2]US Food and Drug Administration, USA.

The triacylglycerol (TAGs) composition of oils and fats is commonly used to assess the authenticity of food materials from which they are derived. Two-dimensional liquid chromatography (2D-LC) has been employed effectively in the past for the separation of complex mixtures of TAGs of plant and animal origin. These separations have most commonly involved the use of normal phase (i.e., nonpolar eluent) separation conditions in one dimension, followed by reversed-phase (i.e., polar eluent) separation conditions in a second dimension. In this presentation we will re-visit the topic of 2D-LC separations of TAGs with a fresh perspective in mind. Specifically, we will discuss the potential of 2D-LC separations of TAGs that leverage recent advances in both optimization theory and instrumentation, and involve reversed-phase separations in both dimensions. We will also examine the effect of column temperature and stationary phase chemistry on selectivity for TAGs in the context of 2D-LC. Finally we weill compare the performance of fully comprehensive (LCxLC) and selective comprehensive (sLCxLC) 2D-LC separations with respect to groups of TAG compounds that tend to overlap even in highly optimized one-dimensional liquid chromatography separations.

Supercritical Fluid Chromatography Utilizing a Quadrapole Time of Flight Mass Spectrometer (SFC QTOF) for the Evaluation of Lipids and Non-polar Molecules. M. Evenson and J. Godbey, Dow AgroScience, USA.

Super critical chromatography has been around since the early 1960's. SFC is a type of chromatography that is old and yet it is new again with the advent of more reliable and easier to use instrumentation. It is similar to normal phase chromatography and the elution order therefore is orthogonal to reverse phase separations. In our studies utilizing the QTOF Mass Spectrometer we have been evaluating columns and mobile phase conditions for lipids and other small molecules found in lipids such as Tocopherols and Tocotrienols. This separation technique along with the QTOF can help

with identification and relative quantification of those compounds that may be difficult to separate with typical Liquid Chromatography or with Gas Chromatography techniques.

Resulting data hints at separation mechanisms with different column types and this data should provide starting conditions for many typical lipids needs.

ANA 3: Olive Oil Analysis

Chairs: R.J. Mailer, Australian Oils Research, Australia; and M. Woodman, Agilent Technologies Inc., USA

Changes in Chemical Composition of Virgin Olive Oil Under Different Cooking Conditions. S. Wang[1], D. Flynn*[1], X. Li[1], and M. Flynn[2], [1]University of California Davis, USA, [2]Miriam Hospital and Brown University, USA.

The currently available studies that assess the effect of heat on extra virgin olive oil utilize cooking temperatures and times that would exceed normal home-cooking conditions. We tested the effect of temperatures and cooking conditions reflective of home-cooking on the chemical compositions, including content of total phenols, individual phenols (e.g. oleocanthal, a-tocopherol and hydroxytyrosol), polar compounds, fatty acids, peroxides, UV absorbanc in olive oils with a range of total phenol content.

Olive oil with very low (10mg/kg), low (140mg/kg), medium (300mg/kg), and high (450mg/kg) total phenol content was heated to 121°C and 180°C on a stovetop for 10 minutes and baked in an oven for 30 minutes at 204°C. After cooling, the oils were analyzed and some of the results are summarized in the table below.

Loss of total phenol content in extra virgin olive oil is lower for stove top cooking at common home cooking temperatures (121°C) and for baking (204°C) compared to frying temperature (180°C). Air exposure during the frying process may be responsible for the higher losses when compared with baking. Oleocanthal, an anti-inflammatory compound, is relatively heat resistant.

Plasticizers as Process Contaminants: A Challenge for Food Oils. Preliminary Studies on DEHP in Olive Oil. P. Miller[1] and C. Guillaume[2], [1]Australian Olive Association, Australia, [2]Modern Olives Laboratory Services, Australia.

There are numerous possible contaminants of fatty foods. These include plasticizers also known as phthalates (ref. Lacoste, 2014, Undesirable substances in vegetable oils: anything to declare?, OCL 2014, 21(1) A103 – www.ocl-journal.org). These lipophilic substances occur at various levels in many plastics and can readily transfer to fatty foods such as oils including olive oil.

Di-2-ethylhexyl phthalate or DEHP is a potential contaminant of food oils.

The Australian laboratory Modern Olives Laboratory Services (MOLS) has conducted preliminary research on DEHP in olive oils to study the level of DEHP in olives oils in the marketplace, and the levels of DEHP that may transfer to olive oils from contact with plastics throughout the processes of growing and harvesting the olives, extracting the oil, storing the oil and then packaging the oil. The levels of DEHP found in randomly selected retail olives oils in the marketplace were between 0.5 and 3ppm.

Many points in the processes from growing to packaging the oils appear to have the potential to contaminate olive oil with DEHP to levels above the limits of 1.5ppm imposed in China and the EU. The potential for contamination varied throughout these processes.

Pyropheophytins and Diacylglycerols as Indicators of Extra Virgin Olive Oil Freshness, Quality, and Authenticity. L. Ravetti, C. Guillaume, N. Ruiz, and D. Zaparenkov, Modern Olives, Australia.

The effectiveness of existing and new analytical parameters such as 1,2-Diacylglycerols ratio (DAGs) (ISO 29822) and Pyropheophytins a (PPPs) (ISO 29841) to monitor the thermo-oxidative and hydrolytic changes during aging of olive oil from different cultivars and Australian regions under various storage conditions has been evaluated. The oils' changing quality was examined through several physico-chemical methods and sensory analysis over 24 months. PPPs and DAGs showed very good performance as indicators of overall olive oil quality and freshness as well as highlighting any problems during the storage of the product. Pyropheophytin a increment over time averaged 7 % per year and the 1,2-diacylglycerols decreased at an average of 23% per year under normal storage conditions. While PPPs has previously been demonstrated to be a good indicator to detect deodorised olive oils, its combined use with DAGs could be also used as an effective measurement of the oils' ageing and storage conditions that those oils have been exposed to. The evolution of these values is highly predictable if storage conditions are known. There was no evidence of varietal or environmental influence on those tests under Australian conditions.

The Effect of Storage Conditions on Olive Oil Quality. J.G. Ayton[1], R.J. Mailer*[2], and K.G. Graham[1], [1]Wagga Wagga Agricultural Institute, Australia, [2]Australian Oils Research, Australia.

The conditions in which olive oil is stored can have an effect on the quality of the oil when it reaches the consumer.

This study evaluated the effect of different storage conditions on a number of olive oils produced in Australia. Samples were exposed to different temperatures, light and oxygen, and tested at intervals over a 3 year period to determine the effect these conditions had on the quality of the oil.

Total polyphenols decreased in oils exposed to oxygen and to high storage temperature. As polyphenols are powerful antioxidants, the Rancimat induction time, or shelf life, also decreased significantly over time. Oils with less resistance to oxidation, such as those with low polyphenol content and high polyunsaturated fatty acid contents, exceeded the International Olive Council (IOC) limit for peroxide value (20 mEq O_2/kg oil) within 8 months when exposed to oxygen. The same oils exceeded the requirements of the IOC for UV absorbance after only 6 months storage.

The initial composition of the oil had a significant bearing on the quality of olive oil when exposed to different storage conditions. This study shows the importance of storage conditions in maintaining the quality of extra virgin olive oil.

A Rapid Method to Evaluate Extra Virgin Olive Oils Quality from Near-UV vis Absorption Spectral Analysis. C. Lazzerini, D. Ancora, M. Cifelli, C.A. Veracini, M. Zandomeneghi, and V. Domenici*, Università di Pisa, Italy.

This work reports a new approach to extract the maximum of chemical information from the absorption spectrum of extra virgin olive oils (EVOOs) in the 390-720 nm spectral range, where "oil pigments" dominate the light absorption. Four most important pigments, i.e. two carotenoids (lutein and beta-carotene) and two chlorophylls (pheophytin-a and pheophytin-b), are chosen as reference oil pigments, being present in all the reported analytical data regarding pigments of EVOOs. The method allows the quantification of the concentration values of these four pigments directly from the deconvolution of the measured absorption spectrum of EVOOs. The main point of this new method is the fact that in less than a minute, within a fast and simple approach, it is possible to extract quantitative information, through the mathematical analysis of the UV-vis spectrum of untreated samples of oil. Advantages and limits of the method, the reliability of the pigment family quantification will be discussed. Moreover, the method has been tested on more than one hundred EVOOs and several mixtures among EVOOs and different olive and seed oils, in different percentages, showing that the method is able to reveal such type of frauds.

Defining Olive Oil Quality: Ancient Questions Come to Life. Z. Kerem, Hebrew University of Jerusalem, Israel.

Archaeological evidence shows that olive oil was produced as early as 7000 years ago. How did early farmers identify the olive fruit as a good source of quality oil, and how is their selection reflected in the evolution of olive oil and agricultural practices? Our findings show that traditionally, clones were maintained vegetatively and grafted, and that a single clone was selected to be grafted. This may be explained in terms of the quality of the produced oils, which are indeed mentioned in early writings, long before modern chemistry. As olive oil production spread, new flavors and qualities were introduced. Today, olive oil is regaining recognition for its high quality and, as such, attracts fraud. International trade standards were developed to protect consumers, based on the chemical composition and flavor of oils produced traditionally along the northern Mediterranean basin. However, olive oil production has spread to new areas, and new agricultural practices are developed to increase the efficiency of its production. We have shown that by manipulating the levels and timing of fertigation, and the choice of cultivar and area, the composition of the oils can be manipulated. New oils, new flavors and new qualities are now available to consumers around the world, who have learned to appreciate olive oil. New markers for quality will be proposed to account for the global developments.

Fatty Acid Alkyl Esters in Extra Virgin Olive Oil: An Evolving Parameter. R.B. Gómez-Coca, M.C. Pérez-Camino, and W. Moreda*, Instituto de la Grasa (CSIC), Spain.

Olive oil is important in the Mediterranean countries' economy and health. The EU, the main producer and consumer of olive oil, and IOC have quality parameters to classify the oil in categories. Oil quality is directly related to agricultural and manufacturing practices. Fruit quality, ripeness, and processing and storage conditions will determine the quality of the oil. A new quality parameter was

introduced for EVO, fatty acid alkyl esters (FAEE), this parameter is directly related to the quality of the olive fruits. High quality olive fruits will have low amounts of FAAE. FAAE are formed by esterification of free fatty acids with short chain alcohols, mainly methanol and ethanol, yielding methyl and ethyl esters. A relationship between the FAAE concentration and their sensory classification can be established, mainly related to fermentative defects. The FAAE presence in EVO –limits will be lowered by the years- were made assuming that the concentrations were fixed, and they did not evolve over time. After a two-year study on the FAAE concentration under controlled storage conditions, demonstrated that the FAAE concentration does not only change with fruit ripeness, but also over time once the oil is bottled. In this line will also present our research and conclusions on the study of the FAAE kinetics.

ANA 3.1/IOP 3: Algal and Other Non-traditional Oils Characterization

Chairs: L.M.L. Laurens, National Renewable Energy Laboratory, USA; and B.W.K. Diehl, Spectral Service AG, Germany

Radiant Energy vs. Organic Carbon: Algal Lipid Profile Diversity in Relation to Cultivation and Conversion Parameters. B.A. Black[1], E. Christensen[1], T. Dong[1], T. Schaub[2], and L.M.L. Laurens[1], [1]National Renewable Energy Laboratory, USA, [2]New Mexico State University, USA.

On an areal-basis, microalgal lipid yield exceeds that of any terrestrial source and lipid composition can be readily influenced by cultivation methods, which increases the suitability of algal lipids for both commercial products and biofuel production. All microalgae are photosynthetic and use light energy to assimilate atmospheric carbon dioxide to form biomass as autotrophic growth. Some organisms are capable of fermentation of organic carbon, often in the form of sugars. By replacing atmospheric carbon assimilation with an organic source in the absence of light, heterotrophic growth and alternative lipid accumulation pathways are initiated. Advantages of each cultivation method exist for the production of lipids; however, profound distinctions are found in the lipid profile of algal strains grown under different carbon utilization scenarios. We present a comparative lipid analysis for oils derived from algal biomass using a combination of FT-ICR and time-of-flight mass spectrometry for comprehensive characterization. Validation of lipid annotation and functional group identification was compared with traditional quantitative chromatographic methods. We show variation of lipid composition and characteristics that relate to cultivation and conversion scenarios and can predict the utilization of these oils for biofuel and bioproduct synthesis.

Analysis of Marine Dietary Supplement Using NMR Spectroscopy. E. Hatzakis, Pennsylvania State University, USA.

A commercial fish oil supplement is expected to be a mixture of several unsaturated (*n*-3, *n*-6, *n*-9) and saturated fatty acids (SFA) in the form of triacylglycerols (TAG) and diacylglycerols (DAG). The determination of the composition of these compounds and the evaluation of other important parameters, such as the positional distribution of fatty acid chains on the glycerol skeleton and the determination of the *n*-6/*n*-3 ratio by using NMR spectroscopy, rely on the correct assignment of the 1D ^{1}H and ^{13}C NMR spectra By employing sophisticated 2D NMR experiments, such as HSQC-TOCSY, band selective HSQC and semi-selective constant time HMBC we performed a systematic two-dimensional analysis of the various components in fish oil. This analysis offered solid proof and confirmation of earlier assignments based on model compounds and revealed the presence of *n*-1 acyl chains and *trans* fatty acids in remarkable concentrations. In addition, we present for the first time the application of ^{31}P NMR in the analysis of micro-constituents in fish oil supplements, which allows the fast and easy determination of additional minor compounds in fish oils. Quantitative results of various compounds in the supplements, was achieved by integrating the appropriate NMR signals in the spectra.

NMR Spectroscopy, a Rapid Method for Any Lipid Analysis. B.W.K. Diehl, Spectral Service AG, Germany.

NMR as an absolute analytical method is the preferred tool to characterize any source of lipid, rare edible oils for cosmetics or bulk ware for biodiesel and the finished biodiesel, too.

Nondestructive methods as well as measurements after specific derivatisation allow the origin test and the determination of any important parameter in lipid composition and quality. A series of single cell oils and rare edible oils are presented in detail.

Complex Mixture Analysis by FT-ICR Mass Spectrometry for Microalgal Biofuel Applications. T. Schaub[1], N. Sudasinghe[1], J. Jarvis[1], A. Nag[2], L.M.L. Laurens[2], E. Christensen[2], and K. Dadamudi[1], [1]New Mexico State University, USA, [2]National Renewable Energy Laboratory, USA.

Variable biochemical composition of algal biomass from different species and cultivation practices has significant impact on production economics, yield and affects downstream catalytic processing. In particular, the distribution of component lipids, determined at the level of individual molecular distributions, is important to determine downstream processing methodology and identify valuable co-product streams. Ultrahigh-resolution mass spectrometry, such as that offered by FT-ICR MS, provides a means to identify the

molecular profile of lipids in algal biomass and fractions generated by processes developed at NREL. Furthermore, novel molecules discovered by this approach, with multivariate data reduction algorithms, provide additional opportunity for economic improvement. We present a novel elemental composition determination procedure based on the application of iterative atomic constraint scenarios to ultrahigh resolution FT-ICR MS data of microalgal lipid extracts. Constraint conditions are derived from elemental composition of known lipids in the Nature Lipidomics Gateway database and facilitate accurate, rapid and direct elemental composition assignment for several thousand lipids per lipid extract. Elemental composition-to-lipid molecule matching provides an unprecedented view of microalgal lipid composition.

A Simple Method for the Isolation of Fucoxanthin from Brown Algae and Its Antioxidant Activity in 5% Fish Oil-in-Water Emulsion. S.F. Koduvayur Habeebullah[1], S. Alagarsamy[2], and C. Jacobsen*[1], [1]National Food Institute (DTU FOOD), Denmark, [2]Fisheries College and Research Institute, India.

Fucoxanthin, a non provitamin A carotenoid is a yellowish brown pigment found abundantly in brown algae. Along with chlorophyll a it is bound to some proteins and act as a light harvesting and transferring pigment. Fucoxanthin has attracted considerable interest in recent years because of its potent bioactivities. The occurrence of carotenoids and chlorophylls in photosynthetic tissues complicates the isolation of pure fucoxanthin and the current available methods for the isolation of fucoxanthin are tedious with use of many solvents. The present study deals with a simple method for the isolation of fucoxanthin and testing their antioxidant activity both in in vitro assays and in 5% oil-in-water emulsion. The yield of fucoxanthin in this method ranges from 0.1% -0.5% depending on the species and is comparable to other methods. Fucoxanthin showed good DPPH radical scavenging and iron chelating property. However, it showed low reducing power and was poor in inhibition of lipid oxidation in liposome model system. When tested in 5% fish oil in water emulsion in the presence of iron, it showed a pro-oxidative behavior in PV and volatile datas but showed a low tocopherol loss compared to control and BHT.

Aggregation Characteristics of Rhamnolipid Biosurfactants and Their Synthetic Variants. R.J. Eismin, R. Palos-Pacheco, C.S. Coss, R. Polt, R.M. Maier, and J.E. Pemberton, University of Arizona, USA.

Rhamnolipids are of current interest as greener alternatives to common synthetic surfactants. However, little is known about the fundamental aggregation properties of these surfactants. To better understand these aggregation properties, surface tensiometry, dynamic light scattering, and static and time-resolved fluorescence spectroscopy were applied to aggregates formed from the native monorhamnolipid mixture produced by *Pseudomonas aeruginosa* ATCC 9027 and to aggregates formed from four diastereoisomers (R-,R-; R-,S-; S-,R-; and S-,S-) of the most common, naturally-occurring monorhamnolipid congener, Rha-C10-C10, produced by chemical synthesis. For concentrations above the CMC of ~180µM at pH 8, globular micelles of ~2-3nm predominate along with two minor aggregates with dimensions of ~12 and ~80nm. Monorhamnolipid micelles were further probed in fluorescence quenching studies to determine average aggregation number of these aggregates. Micelles from these monorhamnolipids have aggregation numbers of ~25-30 molecules/micelle.

On the Use of Microfluidics to Study the Early Formation and Subsequent Stability of Emulsion Droplets. C.C. Berton-Carabin, K. Muijlwijk, and K. Schroën, Wageningen University, The Netherlands.

To control the end-properties of food emulsions, it is necessary to characterize the parameters that affect their early formation and ageing. Emulsions can be prepared using various instruments, for example high pressure homogenizers. Such devices typically induce the break-up of emulsion droplets in the millisecond range. However, most instruments available to study the formation of oil-water interfaces, and notably the adsorption of emulsifiers, hardly give information on the mechanisms occurring at sub-second timescales.

Our work describes the use of microfluidic devices to measure the dynamic interfacial tension between oil and water at the millisecond scale. Using a Y-shaped junction, the mechanism of oil droplet formation was described by relating the shear forces exerted by the aqueous phase, and the oil-water interfacial tension, to the droplet size. A range of static interfacial tensions and shear rates

were used to build a model, which was subsequently applied to estimate the dynamic interfacial tension of droplets formed with different types and concentrations of emulsifiers. We also used microfluidic devices to study the coalescence of emulsion droplets, and identified a range of conditions under which coalescence is favored.

ANA 4a: Advanced Data Analysis

Chairs: T. Haines, Archer Daniels Midland Co., USA; and S. Seegers, Bunge Oils Inc., USA

Automated Fatty Acid Analysis of Edible Oils. G. Jackoway and M. Sasser, MIDI, Inc., USA.

Fatty Acid Analysis of edible oils yields critical information concerning quality, purity, and health benefits. To comply with AOCS Official Methods requires considerable operator time; and subjective analysis may lead to human error. In this presentation, an automated method for either Gas Chromatography (GC) or Gas Chromatography-Mass Spectroscopy (GC-MS) is described. This method assures system suitability by application of response factors, system repeatability by use of a complex external calibration mixture, and has demonstrated inter-laboratory reproducibility. The method automatically calculates Equivalent Carbon Length (ECL) values, presenting results in weight percent and/or mole percent. Further calculations may include iodine value, saponification value, and summation of similar FAME compounds (*e.g.* omega-3, and omega-6 FAMEs). Data visualizations include two-dimensional plots and dendrograms. Databases of stored analyses can be automatically searched by pattern matching algorithms for determination of oil type. The same approach is being applied to the analysis of sterols, triterpenes, glycerides, and tocols, and can be used for detection of adulteration. Automation of analysis and data handling minimizes operator time, thereby reducing costs and improving reliability of results.

Combined Analysis of Stable Isotope Ratio, ^{1}H NMR Spectrum, and Fatty Acid Profiles to Distinguish the Authenticity of Sesame Oils. J. Kim, G. Jin, H.S. Chun, S. Ahn, and B.H. Kim, Chung-Ang University, Republic of Korea.

This study aimed to distinguish the authenticity of sesame oils using combined analysis of stable isotope ratio, ^{1}H NMR spectrum, and fatty acid profiles of the oils. The analytical data were obtained from 35 samples of authentic sesame oils and 29 samples of adulterated sesame oils (25 samples of sesame flavored oils and 4 samples of commercial sesame oils which deviated from the specifications and standards of sesame oil in Korea Food Code) that are distributed in Korea. Seven discriminant variables that most effectively characterize the authenticity of sesame oils were selected by canonical discriminant analysis. The variables include carbon stable isotope value (δ^{13}C), integration values of NMR peaks which signify the terminal CH_3 of 18:3*n*-3 and H5' of sesamolin, respectively, and content values of 18:1*n*-9, 18:3*n*-3, 18:2*t*, and 18:3*t*. The authenticity of all the 17 blind samples of sesame oils was correctly characterized by applying the range of the seven discriminant variables for the authentic sesame oil. These results suggest that the triple analysis is a useful approach for the characterization of sesame oil authenticity.

Purdie Assay: A Novel, Facile, and Cheap Assay for a Wide Array of Applications in Lipid, Terpene, and Estrogen Analyses. G. Dumancas[1], M. Muriuki[2], N. Purdie[2], and R. Purdie[2], [1]Oklahoma Baptist University, USA, [2]Oklahoma State University, USA.

Over the years, our laboratory has developed a novel, facile, and cheap method for the direct determination of lipids (monounsaturated fatty acid (MUFA)/polyunsaturated fatty acids (PUFAs)), terpenes, and estrogens. In this talk, we will provide an overview of the capability of the accomplishments that the “Purdie Assay” has done over the years. We will provide a proof-of-concept of the possible application of the assay for the simultaneous determination of lycopene and beta-carotene in tomatoes, terpenes in terpene-related mixtures, and unsaturated fatty acids in a wide array of insects. These methods are considered to be faster and cheaper than the traditional high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC-MS) methods. This study shows the wide possible applications of the assay as a novel, fast, and efficient tool for lipid quantification and classification.

Application of Chemometric Analysis to the Rapid Screening of Extra Virgin Olive Oils for Authenticity: Evaluation of the Performance of a Handheld NIR Device. S.R. Karunathilaka[1], H. Azizian[2], J.K.G. Kramer[3], and M.M. Mossoba[1], [1]US Food and Drug Administration, USA, [2]NIR Technologies, Canada, [3]Agriculture and Agri-Food Canada, Canada.

While FDA has jurisdiction over deceptive label declarations often found with adulterated extra virgin olive oil (EVOO), the Agency is also mandated with the protection of the US public against the intentional adulteration of foods. A priority for FDA has been the development of novel, rapid, non-

targeted screening tools for the detection of economically motivated adulteration (EMA) of food products such as EVOO based on rapid, high-throughput methods and instrumentation including portable or hand-held devices for field use. The performance of a handheld NIR device was evaluated and compared to that of a benchtop FT-NIR spectrometer. Univariate and multivariate statistical analyses of observed NIR spectra were used to screen commercial products that were labeled extra virgin olive oil (EVOO), and provided a rapid and presumptive indication of authenticity.

ANA 4b: Rapid Methods

Chairs: B. Musselman, IonSense, USA; and H. Adams, Archer Daniels Midland Co., USA

What Does It Takes to Be a Rapid Method? B. Musselman, IonSense, USA.

Abstract not available.

NMR Spectroscopy for Quality Control and Provenience of Olive Oil. B.W.K. Diehl and Y. Monakhova, Spectral Service AG, Germany.

Since olive oil is one of the most affictitious edible it is a need to provide fast and reliable analytical methods to distinguish between original and adulterated oil, its freshness by analyzing the degradation and oxidation products and its provenience by its molecular fingerprint.

^{1}H NMR provides the real peroxide value, aldehydes, free fatty acids, sterols, polyphenols and terpenes, 1,2 and 1,3 DAG, fatty acid composition, squalene and its peroxides. In addition PCA analysis of specific markers of olive oil and its region selective fatty acid distribution enables a reliable provenience test within 10 minutes of measuring time.

Calibration of a Fluorescence-based Sensor and Non-invasive Rapid Method for Detecting Anti-oxidants and Maturation in Tobacco Leaf. E. Bargiacchi[1], M. Campo[2], A. Romani[2], and S. Miele[1], [1]Consortium INSTM, Italy, [2]University of Firenze, Italy.

Non-invasive, rapid methods based on portable sensors can simplify repeated analyses of field crops on a large scale, provided that a previous, careful calibration phase has been carried out. To investigate tobacco anti-oxidants and on-going maturation, a fluorescence-based sensor (Multiplex®, ForceA-France) was used, comparing this sensor lab and field measurements with HPLC/DAD/MS analyses of the hydroalcoholic extracts of the same tobacco leaves, for calibration. Tobacco was analyzed for flavonols, nitrogen, nicotine, and nicotine derivatives at different stages, both in variety tests, field experiments and at growers' fields (Fattoria Autonoma Tabacchi, Italy) in 2013-2014. Mx indices were calculated for polyphenols (FLAV), chlorophyll (CHL), and total nitrogen (NBI). FLAV was measured at 375 nm wavelength, and calculated as log (FRFr/RFRuv). Correlation (r^2) between FLAV indices and destructive analyses was 0.7-0.8. Results indicated that this sensor can be used on fresh and cured tobacco leaves. Further investigations will account for variety differences, and some major agronomic aspects (e.g. type of irrigation). On our knowledge, this is the first time this equipment is used on tobacco. This research, was in part funded by Regione Umbria QUALITABA Project to improve tobacco quality.

Application of NIR and Chemometrics for the Untargeted Screening of Extra Virgin Olive Oil for Assessment of Authenticity. M.M. Mossoba[1], S. Ranasinghe[1], H. Azizian[2], A. Fardin-Kia[1], J.K.G. Kramer[3], C.T. Srigley[1], P. Delmonte[1], and J. Callahan[1], [1]US Food and Drug Administration, USA, [2]NIR Technology, Canada, [3]Agriculture and Agri-Food Canada, Canada.

Economic adulteration of extra virgin olive oil (EVOO), the highest grade of olive oil, is a threat to its authenticity. Cases of fraud of EVOO have long been reported by the media and in the scientific literature. The most serious issue facing regulatory agencies has been the relative ease to adulterate EVOO with lower grade olive oils or seed or nut oils while meeting the physical and chemical property limits of various established Standards. Near-infrared (NIR) spectroscopy (using benchtop spectrometers and/or portable analyzers) and univariate and/or multivariate statistical analyses were applied to the rapid screening of 93 commercial EVOO purchased locally. Other untargeted and targeted complementary analytical tools and/or discrimination algorithms were also used. Depending on analytical methodology used approximately 5-20% of the oils were flagged as outliers. Adulteration at 10% and 20% (w/w) of an authentic EVOO reference with 10 different foreign oils, including palm olein and hazelnut oil, was also detected.

Statistical Modeling of Data from Intact Triglycerides and Their Degradation Products for Rapid Assessment of Milk Quality and Authenticity. B. Musselman[1], R. Goguen[1], C. Hart[2], and J. Lapointe[1], [1]IonSense, Inc., USA, [2]Boston University Forensics, USA.

Milk is a major food source in the world and has

recently been a target of adulteration. Rapid determination of authenticity and detection of contaminants has been shown using Principle Component Analysis (PCA) of direct analysis in real time (DART) mass spectrometry data (1). In the PCA studies samples were prepared by extracting non-polar triglycerides from the milk of different animal species. In short, wide variations in the TAG distribution were present. In our investigation, we focused on analysis of closely related whole and skim cow milk where TAG variations were potentially minimal. Differences in mass spectra were much less pronounced in the same animal species was smaller and, thus, the PCA method was not useful.

In this study, milk was diluted to 1% in water and multiple 3ul aliquots were analyzed directly using a high resolution Orbitrap mass spectrometer. Statistical models were built using a variety of statistical classification methods. Those models were tested against a set of unknowns collected on a different day to give the testing error rates. The application of a Support Vector Machine model proved most suitable for rapid sample classification.

Hrbek, et al. Food Control, 36, (2014) 138-145.

ANA 5a: Advances in *trans* Fat Analysis

Chairs: S.D. Bhandari, Silliker, USA; and M.M. Mossoba, US Food and Drug Administration, USA

Advances in the Analysis of *trans* EPA and DHA in Fish Oil Supplements. C.T. Srigley, US Food and Drug Administration, USA.

Fish oil (FO) supplements are among the most frequently consumed dietary supplements in the United States (US) and other countries. These products have as their intended purpose, among others, the reduced risk of coronary heart disease due to their contents of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). FO supplements have also been shown to contain *trans* isomers of EPA and DHA that are formed as a result of the geometrical isomerization of all-*cis* EPA and DHA during high temperature processing. This work evaluated the content and composition of the *trans* isomers of EPA and DHA from the wide variety of FO supplements currently available in the US. Fatty acid methyl esters were analyzed by gas chromatography with flame ionization detection on a 200 m SLB-IL111 ionic liquid column. Combined concentrations of the *trans* isomers of EPA and DHA were as high as 1.5% of total fat, corresponding to a mean content of 8 ± 10 mg/serving and a mean estimated intake of 10 ± 11 mg/d based on label directions for product use. The nutritional significance of the contents of the *trans* isomers of EPA and DHA in currently available FO supplements remains to be determined.

Analysis of *trans* Fat by GC, Silver Ion TLC-GC, and Silver Ion-HPLC. C. Cruz-Hernandez and P.A. Golay, Nestlé Research Center, Switzerland.

The identification of *trans* fatty acids (TFA's) in different foods, mainly industrially produced *trans* fats, has been of great interest in recent years as these FA's have been related to increased risk of coronary heart disease (CHD). Conversely, some sources of edible fats, such as ruminant fats, contain a wide variety of FA's, some of which are associated with unique health benefits. Fats from various sources and processes can differ not only in the amount of *trans* fat but, more importantly, in the relative distribution of the different positional isomers. Several good methods are now available for the determination of TFA's including recent developments in chromatographic techniques for their analysis. This work is intended to show the major techniques of *trans* fat analysis including the process of extracting lipid constituents and methylation. The various separation methods include thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC) and argentation (silver ion, Ag+) chromatography on TLC or HPLC phases. Some of the most recent methods to determine *trans* FAME describe separations that require long capillary columns with highly polar stationary phases. Typical FAME profiles for total milk fat and partial hydrogenated vegetable oils will be shown according to the technique performed for the analysis.

Portable Infrared Sensors for Determination of *trans*-fat Content in Food Products. L.E. Rodriguez-Saona and M. Plans Pujolras, Ohio State University, USA.

In order to comply with FDA regulatory requirements for *trans*-fatty acids, it is required a fast, accurate, easy-to-use analytical methods for fats, oils, and the products that contain partially hydrogenated oils. We report on advances in portable infrared spectrometers to assess *trans*-fat content in a variety of commercial food products. Samples (cakes, doughnuts, cookies, potato chips, etc) obtained from various grocery stores were evaluated for *trans*-fat using partial least squares regression and linear regression using the negative second derivative band height at 966 cm^{-1}, associated with the *trans* CH out-of-plane deformation vibration. GC analysis was used as reference method to evaluate predictive accuracy of the models. Although oils in bakery foods required extraction with organic solvents before analysis, the method was rapid (~2 min) for determination of *trans*-fat and showed very good predictive accuracy (R>0.97) when compared with GC values. Multivariate analysis for portable IR units gave standard error of prediction (SEP) of 0.5%, and our data showed some prevalence of under-representation of *trans*-fat levels in labels among samples. Portable ATR-IR units combined with chemometrics can provide food manufacturers reliable methods to measure *trans* fat content to determine compliance with food nutrition labeling laws.

Analysis of *trans*-fatty Acids in Human Plasma, Serum, and Red Blood Cells by Isotope Dilution GC-MS Using Negative Chemical Ionization. H.C. Kuiper, N. Wei, S.L. McGunigale, N. Ahuja, T. Frame, and H.W. Vesper, Centers for Disease Control and Prevention, USA.

Trans-fatty acid (TFA) intake is associated with increased risk of cardiovascular disease. Studies suggest that individuals with the same fatty acid (FA) intake may have different concentrations of FA in plasma, serum, and red blood cells (RBC) due to many factors related to metabolism and lifestyle. Little is known about TFA concentrations in human blood. This created the need to assess TFA concentrations in different human blood specimens to obtain information about TFA exposure and metabolism. The low proportion of TFA to total FA in most specimens requires a sensitive analytical method with a large dynamic range. With the complexity of biological samples, including positional and geometric FA isomers, the analytical method must also be highly specific. We have developed and validated an isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry method that enables the assessment of 4 major TFA and 24 other FA in 100µl of human blood at levels as low as 0.05µM. We investigated TFA levels in plasma, serum, and RBC from 66 convenience samples and TFAs were detected in all samples, with median total TFA concentrations of 21.12µM in RBC, 17.86µM in plasma, and 20.88µM in serum.

ANA 5b: Sample Pretreatment/Handling

Chairs: K. Persons, Eurofins Scientific Inc., USA; and M. Evenson, Dow AgroSciences, USA

Novel Rapid Method for the Determination of Frying Oil Quality Based on Capillary Penetration. E.P. Kalogianni, D. Georgiou, and A. Marinopoulou, Alexander Technological Educational Institution of Thessaloniki, Greece.

A novel rapid method under development for the determination of frying oil quality is presented. The method is based on a completely different principle than the ones already existing: the measurement of capillary penetration rate of the oil (or fat) in a substrate. Under certain conditions, the penetration rate is affected only by the oil viscosity and surface/interfacial tension. Both viscosity and surface/interfacial tension change during frying with viscosity even doubling close to the oil rejection limit. Furthermore, existing regulation criteria in several countries include the concentration of polymer and polar compounds. Polymer and polar compounds concentration relate to the changes in viscosity surface/interfacial tension in the oils during frying. The method is simple and inexpensive and completes within several seconds up to a few minutes time. It can provide quantitative and qualitative results. The method is aimed both for quality control labs in the food industry but also for the catering sector including fast foods because it is safe for the foods being prepared in the same area and simple to use for non-specialized personnel. The present work presents the principle of the method, the design and prototype of the apparatus and results for fresh, heated and fried oils.

Oxidative Stability of Cashew Nut (*Anarcardium occidentale*) Oil. M. Azih, Ambrose Alli University, Nigeria.

The stability of cashew nut oil against oxidative stability was tested in this study. Oil was extracted from sun-dried freshly harvested cashew nuts using the Folch method. The oil was exposed to the atmosphere at room temperature (25°C) for 20 days and measurements of iodine, peroxide and TBA values were taken at 5-day intervals. The highest increase (121%) was recorded in the TBA value over the 20-day period, while the increase in peroxide value was 48%. The iodine value decreased by 44%. The trend observed is believed to be a consequence of the fatty acid profile of cashew nut oil and the unique molecular events of each parameter measured. The results suggest a predisposition of cashew nut oil to oxidative rancidity at room temperature, and a consequent need for specialised storage conditions in order to maintain product stability.

A Novel Method for the Automatic Sample Preparation and Analysis of 3-MCPD-, 2-MCPD- and Glycidylesters in Edible Oils and Fats. R.P. Zwagerman and P.M. Overman, IOI Loders Croklaan, The Netherlands.

Currently, there are three recommended AOCS indirect methods for the detection and quantification of 2- and 3-chloropropane-1,2-diol-(2-MCPD/3-MCPD) and glycidyl esters. The analysis is time consuming and because of extensive manual sample preparation, the chance of human error is significant and well-trained analysts are required for correct and reproducible results.

We developed a new automated indirect method for sample preparation and quantification of these three analytes in oils and fats using GC-MS/MS based on AOCS Official Method Cd 29c-13. The method is adapted to ensure separate glycidol detection and correction for possible overestimation due to conversion of 3-MCPD to glycidol during alkaline transesterification, using a Carbon-13 labeled internal standard. Furthermore, the total analysis time is reduced significantly to less than five hours per series of four samples with minimal contact time. The exclusion of manual sample preparation reduces the need for dedicated well-trained analysts and improves reproducibility. This makes the automated method a suitable tool to implement in operational Quality Control services without specialized analysts. The method has been validated against AOCS Official Method Cd 29b-13 using different types of refined edible oils.

A Method for Isolation and Lipid Characterization of Chicken Yolk Vitelline Membranes. S. Shinn, R. Liyanage, J. Lay, E. Martin, and A. Proctor, University of Arkansas, USA.

The vitelline membrane (VM) separates the yolk from the albumen in chicken eggs and is composed of two layers with a continuous membrane in between. The VM proteins and carbohydrates have been characterized, but lipid content reports are inconsistent and information on VM lipid composition is unavailable. The objectives of this

study were to 1) determine a VM washing and isolation protocol 2) compare the VM total lipid content from the Folch and hexane:isopropanol extraction methods and 3) determine the fatty acid and total lipid composition of the VM. Quadruplicate pooled samples of 15 membranes will be isolated by 4 different methods: 1.) quadruplicate water washes (Britton 1973), 2.) duplicate water wash, followed by ether:ethanol (1:3 v/v) wash, and final water wash (Bellairs, 1963) 3.) water wash and sonication 4.) water and chloroform wash. Duplicate isolated membranes were extracted by either the Folch Method or hexane:isopropanol method. Lipid extracts were converted to FAMEs for fatty acid analysis. SEM was used to observe any changes in the membrane following each washing treatment. To determine lipid composition both sides of triplicate isolated membranes from the most satisfactory washing method were analyzed by MALDI-TOF-MS.

ANA-P: Analytical Poster Session

Chairs: T. Mason West, Bunge Oils, Inc., USA

1. **Simultaneous Determination of Cholesterol and Monounsaturated/Polyunsaturated Fatty Acids Using a Novel Assay, Clustering Algorithms, and Genetic Algorithm Partial Least Squares.** G. Dumancas[1], M. Muriuki[2], N. Purdie[2], and R. Purdie[2], [1]Oklahoma Baptist University, USA, [2]Oklahoma State University, USA.

Current methodologies of quantifying cholesterol and monounsaturated fatty acid (MUFA)/polyunsaturated fatty acids (PUFAs) constitute techniques such as GC and HPLC requiring significant analysis time. We sought to develop a simple, direct alternative method for the simultaneous determination of cholesterol and MUFA/PUFAs (linoleic linolenic, arachidonic, eicosapentaenoic, docosahexaenoic, conjugated linoleic, and oleic acids) using a novel "Purdie Assay", clustering algorithms, and genetic algorithm partial least squares (GAPLS). The assay with GAPLS simultaneously quantified these lipids without any need for analytical separations in human serum and vegetable oils. Using the assay, we performed pattern recognition of biological and food samples using principal component analysis (PCA) and hierarchical clustering (HC). The assay successfully discriminated 11 clusters corresponding to different food and biological samples. The assay was also able to discriminate synthetic vegetable oil samples using PCA and HC corresponding to levels of lipids prepared. This study shows the wide possible applications of the assay as a novel, fast, and efficient tool for lipid quantification and classification.

2. **Drying Seeds High in Polyunsaturated Fatty Acids Can Lead to Erroneous Results When Drying to Constant Weight.** M.M. Al-Amery, S. Patel, M. Ma, M. Sanches, T. Phillips, P. Armstrong, and D. Hildebrand, University of Kentucky, USA.

Oilseeds are important sources of oils high in polyunsaturated fatty acids that are useful for edible and industrial purposes. For health and nutrition there is particular interest in oils high in ω-3 fatty acids. It is useful to determine the moisture content of oil sources for total material analysis and to calculate levels of oil, specific fatty acids. A standard method for determining moisture content and dry weight is to place samples at temperatures > 100°C until the weights stop changing. We find with both soybean and chia (*Salvia hispanica*) seeds extensive lipid oxidation can occur with such drying resulting in weight increases with oxygen incorporation into lipid oxidation products and weight loss from volatile secondary lipid oxidation products along with aberrant oil measurement. This problem is greater with ground than whole seeds. Carefully following AOCS and NIST seed drying protocols can minimize such erroneous results when drying is stopped at the prescribed times. Drying in an atmosphere of nitrogen (N_2) with constant N_2 flow can eliminate lipid oxidation but results in lower final dry weights and correspondingly 1- 2% higher estimated moisture contents than drying in regular atmospheres with normal oxygen levels.

3. **Development of a Rheo-NMR+XRD System Prototype for the Advanced Photon Source Synchrotron.** G. Mazzanti[1,2], S. Weigand[3,4], J. Rix[3,4], X. Deng[1], Y. Wang[1], P.K. Batchu[1], A.W. Alkhudair[1], R. Liu[1], O. Qatami[1,2], L. Lin[1], and T. Jia[1], [1]Dalhousie University, Canada, [2]Institute for Research in Materials, Canada, [3]Argonne National Laboratory, USA, [4]Advanced Photon Source, USA.

Fats are multicomponent lipid materials made up mainly of triacylglycerols (TAGs). During the industrial processing, the quality of an end product (i.e. its) is determined by the processing conditions that affect the crystallization kinetics. However, the kinetics are not fully understood and therefore the distribution of phases is generally unpredictable. To build predictive models we need quantitative time-resolved data on phase distributions obtained in-situ under different processing conditions. The integrated intensity from XRD is related to the mass of the phase via proportionality factors. These factors are derived from correlations between XRD data and the solid fraction (SF) measured by NMR. After determining the proportionality factors, phase distributions can be quantified. There were no methods to correlate the SF with the integrated intensities. The first of its kind, time-resolved experiments simultaneously acquiring rheology, XRD and NMR data were conducted at Beamline 5-ID-D at the Advanced Photon Source, Argonne National Laboratory, Argonne, IL. Mixtures of tristearin in tributiryn, and tripalmitin in tributirin were crystallized under shear flow and diffraction patterns

were captured by three detectors at small, medium and wide angles.

4. **A Wireless Communication Contact Closure System for Four Mass Spectrometers and Two Liquid Chromatographs in Parallel (LC2/MS4).** W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA.

The construction of a wireless communication contact closure signaling system, using newly available electronic components, is described that allows the contact closure start signals from either of two liquid chromatographs to be sent wirelessly to four mass spectrometers and several other detectors, liquid chromatography pumps, and syringe pumps. This new system allows a wide variety of experiments to be performed using two liquid chromatographs, four mass spectrometers, two analog-to-digital converters, an evaporative light scattering detector, a corona charged aerosol detector, and two syringe pumps. Experiments can be reconfigured using only the flips of a few switches to change from multiple parallel mass spectrometry experiments, such as liquid chromatography with 'quadruple parallel mass spectrometry', a so-called LC1/MS4 experiment, to column-switching experiments using two liquid chromatographs and up to four mass spectrometers, LC2/MS4. Additional control of external electronically controlled switching valves involved in column-switching experiments and fraction collection is demonstrated.

5. **Diversity of Enzyme/Substrate Ratios and Duration of Exposure Time are Related to Variability of Hydrolysis of Lipoprotein Oxo-PtdChos by Secretory Phospholipases A2 ($sPLA_2$s).** A. Kuksis and W. Pruzanski, University of Toronto, Canada.

$sPLA_2$s have been generally known to hydrolyze arachidonoyl GroPCho to provide substrate for biosynthesis of PGE_2 and LTB_4, which are pro-inflammatory and pro-atherogenic, and much effort has been expended to inhibit their activity. We have recently shown that $sPLA_2$s also hydrolyze PtdCho hydroperoxides, hydroxides and isoprostanes of lipoproteins releasing oxo-fatty acids and lysoPtdChos. We have suggested that $sPLA_2$s may participate in repair of lipid membranes, which have become distorted by oxo-PtdChos. The present study shows that the release of oxo-fatty acids from lipoprotein PtdChos is more complex than that reported for oxo-PtdCho liposomes. When the rates of hydrolysis of the various lipoprotein oxo-PtdChos were compared on a mole/mole basis to those of their parent molecules using $sPLA_2$s (groups IIA, V and X), high enzyme/substrate ratios and long incubation times favored the hydrolysis of oxo-PtdChos, while low enzyme/substrate ratios and short incubation times favored the hydrolysis of native PtdChos.It is not known how the above enzyme activity is related to the massive release of group IIA $sPLA_2$ during inflammation.

6. **Interactions Between Food Gums and Soy Flour.** T.W. Hou[1,2], H. Zhang*[2], Y.L. Bi[1], and X.B. Xu[2], [1]Henan University of Technology, China, [2]Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd., China.

To investigate the interactions between gums (CMC,?-carrageenan and xanthan) and soy flour, solutions with gums and soy flour were prepared and characterized. The viscosity, turbidity, electron energy spectrum and infrared spectrogram of samples were evaluated. Results revealed that soy flour (4%)showed synergism with xanthan (0.2%) as shown by the increase of viscosity of mixed system, flocculation occurred due to the formation of hydrogen bonds between xanthan and soy flour. Soy flour (4%) also showed synergism with?-carrageenan (0.2%), flocculation occurred due to the hydrogen bonds between?-carrageenan and soy flour. Besides, the presence of K^+ promoted the formation of gel. Soy flour (4%) showed antagonism with CMC (0.2%) as shown by the decrease of the viscosity of mixed system and phase separation occurred, due to the electrostatic repulsion between CMC and soy flour.

7. **Preliminary Studies on the Nanostructure of Milk Fat.** P.R. Ramel and A.G. Marangoni, University of Guelph, Canada.

Through characterization using small-angle X-ray diffraction (SAXD) and visualization by cryogenic transmission electron microscopy (Cryo-TEM), the nanoscale structure of milk fat crystal networks, as never before, was described.

Revealing this supramolecular structure by breaking down compound crystals into nanoplatelets allowed us to pinpoint specific crystal plane spacings (size), dimensions and organizations in the structure that are most likely responsible for imparting different functional properties to milk fat products. The complexity in the thermal (melting) behavior of milk fat was explained from a concept of a complex mixture of crystal nanoplatelets of different compositions rather than from general polymorphism arguments.

Finding representative structures for the

system, however, proved challenging because of the fact that milk fat is composed of approximately 400 different fatty acids that make-up various triacylglycerols including minor components such as di- and mono-acylglycerols that all aggregate upon crystallization.

Nonetheless, results of the experiment give a fresh perspective on how to affect the functional properties of milk fat products through manipulation of the structure at the nanoscale.

8. **Direct Separation of the Diastereomers of Phosphatidylcholine Hydroperoxide Bearing 13-hydroperoxy-9*Z*,11*E*-octadecadienoic Acid Using Chiral Stationary Phase HPLC.** J. Ito[1], S. Kato[1], K. Nakagawa[1], T. Nagai[2], and T. Miyazawa[1], [1]Tohoku University, Japan, [2]Tsukishima Foods Industry Co., Japan.

Increasing evidence suggests that phospholipid peroxidation plays important roles in the pathogenesis of various diseases, such as atherosclerosis. With regard to the biochemical processes that initiate phospholipid peroxidation *in vivo*, enzymatic conversion of phosphatidylcholine to phosphatidylcholine hydroperoxide (PCOOH) by lipoxygenase may play a crucial role. This will become clear if we can analyze PCOOH bearing hydroperoxy fatty acids with *S*-stereoconfiguration. In this study, we therefore attempted such an analysis. Initially, we prepared PCOOH bearing 13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid. We used chiral stationary phase HPLC, a UV detector and a quadrupole-time-of-flight mass spectrometer, and achieved diastereomer separation of PCOOH stereoisomers with excellent resolution and peak shape. This is the first study reporting the diastereomer separation of PCOOH. The present method will be beneficial in developing a better understanding of the biochemical processes that initiate oxidative stress (PCOOH formation) *in vivo*, which may lead to further elucidation of the involvement of PCOOH in the development of diseases.

9. **Determination of Short Chain Carboxylic Acids in Vegetable Oils and Fats Using Ion Exclusion Chromatography Electrospray Ionization Mass Spectrometry.** J. Viidanoja, Neste Oil Corp., Finland.

A new method for quantification of short chain C1-C6 carboxylic acids in vegetable oils and fats by employing Liquid Chromatography Mass Spectrometry (LC-MS) has been developed. The method requires minor sample preparation and applies non-conventional Electrospray Ionization (ESI) liquid phase chemistry. Samples are first dissolved in chloroform and then extracted using water that has been spiked with stable isotope labeled internal standards that are used for signal normalization and absolute quantification of selected acids. The analytes are separated using Ion Exclusion Chromatography (IEC) and detected with Electrospray Ionization Mass Spectrometry (ESI-MS) as deprotonated molecules. Prior to ionization the eluent that contains hydrochloric acid is modified post-column to ensure good ionization efficiency of the analytes. The averaged within run precision and between run precision were generally lower than 8%. The accuracy was between 85 and 115% for most of the analytes. The Lower Limit of Quantification (LLOQ) ranged from 0.006 to 7mg/kg. It is shown that this method offers good selectivity in cases where UV detection fails to produce reliable results.

10. **Pigments' Content of Extra Virgin Olive Oils from Different European Countries Produced in 2014.** C. Lazzerini, M. Cifelli, and V. Domenici*, Università di Pisa, Italy.

Last fall, from September to November 2014, has been defined one of the worse for Extra Virgin Olive Oil (EVOO) production in Europe. For example, the production of EVOOs from Italian olive oils drop of about 70% with respect to the previous years. Not only the quantity of EVOOs reduced drastically, but also the quality seems to be changed: the worse quality of EVOOs could be related to sensitive changes in the minor components, such as pigments, with respect to previous years. In this work, several EVOO samples from Greece, Tunisia, Italy and Spain, produced in the period September - November 2014, were analyzed: their pigments' content will be reported and discussed in comparison with EVOOs produced in 2013 and 2012. In particular, we focused our attention on the following minor components: β-carotene, chlorofills, pheophytins *a* and *b*, lutein, zeaxantin and cryptoxantin. These pigments were identified and quantified by means of HPLC/DAD. Moreover, some of them were quantified also by using our new method [1] to extract pigments' information from the UV-vis absorption spectra of EVOOs in the 390-720nm spectral range.

11. **Lipid Fraction Extracted from *Centranthus ruber* Seed Contains Conjugated Linolenic Acid.** T. Honma, Y. Banno, and T. Takayanagi, Tokyo University of Technology, Japan.

Extracts from some plants in Valerianaceae families are reported to have antitumor effects. However, the antitumor substance contained in those plants has not been revealed. In this study, we focused on their fatty acid composition and investigated the antitumor substance in lipid extracts from the seeds of plants in Valerianaceae families.

Seeds of several Valerianaceae plants were homogenized and the lipid fractions were extracted from the homogenates using the Bligh & Dyer procedure. The fatty acids in the lipid fractions were methylated with trimethylsilyldiazomethane and sodium methoxide/methanol, and then analyzed by gas chromatography. The lipid fractions were fractionated to neutral lipids, glycolipids, and phospholipids by silica column chromatography. The obtained lipid classes were further separated by thin layer chromatography. The fatty acid compositions in the separated fractions were measured using gas chromatography.

The lipid fraction extracted from *Centranthus ruber* seeds contained a-eleostearic acid (9c, 11t, 13t-18:3), one of the conjugated linolenic acids that were reported to have antitumor effects. Most a-eleostearic acid of *Centranthus ruber* existed as a triacylglycerol.

12. **Distinguishing Edible Oils by Their Thermal Characteristics Using Fast DSC.** I.A. van Wetten[1,2], A.W. van Herwaarden[1], R. Splinter[1], R. Boerrigter-Eenling[3], and S.M. van Ruth[2,3], [1]Xensor Integration, The Netherlands, [2]Food Quality and Design Group Wageningen UR, The Netherlands, [3]RIKILT Wageningen UR, The Netherlands.

Differential Scanning Calorimetry (DSC) is used for distinction of edible oils. We measured a range of edible oils with Fast DSC at a heating and cooling rate of 100°C/s (instead of a few °C/min as in DSC) in the temperature range from -80°C to +60°C, and also with slow cooling at 2°C/s.

In the cooling curve all oils show one major exothermic peak, attributed to crystallization of triacylglycerols, with peak temperatures ranging from -55°C to -1°C and enthalpies up to about 50kJ/kg. The heating curves after slow cooling of peanut, olive and high-oleic sunflower oils show a large cold endothermic peak (in the range of -65°C to -51°C), with enthalpies up to about 16kJ/kg, which is absent after fast cooling. The enthalpy of this peak decreases rapidly with decreasing oleic acid content. Palm and rapeseed oils show a tiny peak, soybean, coconut and corn oils show no peak. There is always a warm endothermic peak, associated with melting, in the range of -29°C to +1°C, with enthalpies up to 50kJ/kg. These differences in thermal characteristics make Fast DSC promising for the distinction of edible oils.

13. **Chemical Characterization of Monovarietal Avocado Oils.** G.D. Fernandes[1], R.B. Gómez-Coca[2], M.C. Pérez-Camino[2], W. Moreda[2], and D. Barrera-Arellano[1], [1]University of Campinas, Brazil, [2]Instituto de la Grasa–CSIC, Spain.

Although the avocado oil chemical composition has been cited in several studies -normally in comparison with olive oil- its chemical composition hasn't been described thoroughly. Therefore, the aim of this work was to characterize the major and minor compounds of several monovarietal avocado oils. Oils from the Bacon, Fuerte, Hass, and Pinkerton cultivars (IHSM-CSIC, Spain) were obtained from the pulp of mature fruits by means of an Abencor® system and an additional centrifugation step. Following official and/or standardized methods the oils composition was determined and content on triacylglycerol, fatty acids, aliphatic and terpenic alcohols, desmethyl-, methyl-, and dimethylsterols, and squalen were obtained. The main triacylglycerols were those with ECN48 and ECN46. Besides, oleic, palmitic, linoleic and palmitoleic acids prevailed. Desmethylsterols were the principal minor compounds, being ß-sitosterol the most abundant one. Citrostadienol and cicloartenol were the main methyl- and dimethylsterols, respectively, and low amounts of aliphatic and terpenic alcohols were also found. Finally, the squalen concentration was higher in Bacon, Fuerte and Pinkerton oils than in Hass. The chemical composition of avocado oil is quite similar to the olive oil regarding the minor compounds.

14. **Compositional Effects on Fat Crystallization within Confectionery Systems.** R. West and D. Rousseau, Ryerson University, Canada.

It is well-established that the fat crystallization pathway in confectionery products depends on its chemical composition, processing conditions, as well as storage conditions. This fat crystalline network plays a critical role in both quality and organoleptic properties of the final product. Here we investigate how fat crystals grow in micro-confined environments and how they grow in

multicomponent systems where other confectionery ingredients are present. Using palm fats relevant to the confectionery and biscuit industry, fat crystal nucleation, growth rate, and rheology are examined. A novel imaging algorithm that uses polarized light microscopy to distinguish multicomponent systems according to their morphology has also been implemented. The information that is gained from this research allows us to establish and optimize strategies for the improvement of commercial products that undergo fat crystallization.

15. **Rapid Identification and Quantification of an Adulterant Oil in Extra Virgin Olive Oil.** M.M. Mossoba[1], H. Azizian[2], S.R. Karunathilaka[1], A. Fardin Kia[1], P. Delmonte[1], C.T. Srigley[3], J.K.G. Kramer[3], and J. Callahan[1], [1]US Food and Drug Administration, USA, [2]NIR Technologies, Canada, [3]Agriculture and Agri-Food Canada, Canada.

A new rapid Fourier transform near infrared (FT-NIR) spectroscopic procedure is described to screen for the authenticity of extra virgin olive oils (EVOO) and to determine the type and amount of an adulterant oil (e.g., soybean oil, palm olein, or refined olive oil) in EVOO. While FDA has jurisdiction over deceptive label declarations often found with adulterated EVOO, the Agency is also mandated with the protection of the US public against the intentional adulteration of foods. To identify the type and determine the quantity of an adulterant, gravimetric mixtures were prepared by spiking an EVOO with different concentrations of each adulterant. Based on FT-NIR spectra, four PLS calibration models were developed for four specific groups of adulterants each with a characteristic FA composition (e.g., high-linoleic acid or high-oleic acid). Using these different PLS calibration models for prediction, plots of predicted vs. gravimetric concentrations of an adulterant in EVOO yielded linear regression functions with four unique sets of slopes, one for each group of adulterants. Four corresponding slope rules were defined that allowed for the determination of the nature and concentration of an adulterant in EVOO products by applying these four calibration models. The standard addition technique was used for confirmation.

16. **Improved Sinigrin Analysis.** D. Yuan[1], Y.Y. Shim[1,2], K. Ratanapariyanuch*[1], V. Meda[1], and M.J.T. Reaney[1,2,3], [1]University of Saskatchewan, Canada, [2]Prairie Tide Chemicals Inc., Canada, [3]Jinan University, China.

An ion-pair reversed phase HPLC method was developed for the analysis of sinigrin in *Brassica carinata* (A.) Braun seed and seed fractions. Separation was performed on an Inertsil® ODS-4 C18 column and an Eclipse ZORBAX Eclipse XDB-CTM 18 column, with an isocratic eluent containing 100% aqueous tetramethylammonium bromide (10mM, pH 5.0). Sinigrin retention was affected by HPLC variables including the type of ion-pair reagent, buffer strength and pH, concentrations of acetonitrile, column temperature, and eluent flow rate. Partial validation demonstrated this optimized chromatographic condition to be linear, accurate, and precise. Sample preparation involved multistage extraction using 70% (v/v) aqueous methanol that was proven to be more efficient than 50% (v/v) aqueous acetonitrile. In addition, the matrix effect, recovery rate as well as processing efficiency of the analytical protocol were determined. This method is suitable for high throughput analysis of sinigrin in *B. carinata* seed and seed fractions.

17. **Authenticity Assessment of Extra Virgin Olive Oils in the United States Market: Evaluation of the Sterol and Triterpene Dialcohol Content and Composition.** C.T. Srigley, C.J. Oles, A.R. Fardin Kia, M.M. Mossoba, and P. Delmonte, US Food and Drug Administration, USA.

Extra virgin olive oils (EVOO) have a long history of economic adulteration, the detection of which is challenging due to the diverse composition of cultivars grown around the world. This study evaluated the authenticity of 93 commercially-available samples of EVOO, virgin olive oil, and olive oil blends by analyzing their sterol and triterpene dialcohol content and composition, where by applying Method COI/T.20/Doc. No. 30 of the International Olive Council and purity criteria established by the United States Department of Agriculture's (USDA) Standards for Grades of Olive Oil and Olive-Pomace Oil. Of the 88 samples marketed as EVOO, five failed to meet purity criteria for adulteration with foreign oil (e.g., campesterol =4.5% of total sterols) or lower grades of olive oil (i.e., erythrodiol and uvaol =4.5% of total sterols). The method was also evaluated by spiking an EVOO sample with commodity oils at the 10% level. Eight of the spiked samples (canola, corn, hazelnut, peanut, safflower, soybean, and sunflower oils, palm olein) failed to meet purity criteria, whereas two samples, both spiked with hazelnut oil, went undetected for adulteration. Overall, our findings indicate a low level of adulteration (~5%) for EVOO

based on criteria for sterols and triterpene dialcohols.

18. **Determination of Bile Acids in Piglet Bile by Solid Phase Extraction and Liquid Chromatography-electrospray Tandem Mass Spectrometry.** S. Mi[1], D.W. Lim[1], J.M. Turner[1], P.W. Wales[2], and J.M. Curtis[1], [1]University of Alberta, Canada, [2]Hospital for Sick Children and University of Toronto, Canada.

Bile acids (BAs) are produced in liver via oxidation of cholesterols. Its dysmetabolism is closely associated with the occurring of some liver diseases, such as parenteral nutrition-associated liver disease (PNALD). The objective of this work was to develop a method for the quantitative profiling of individual BAs in piglet bile and apply this to elucidate the effect of glucagon-like peptide 2 (GLP-2) on BA synthesis and transport. A procedure involving the isolation of BAs directly from diluted bile samples onto C18 solid phase extraction cartridges was developed and optimized. 19 BA standards including glycine and taurine conjugates were simultaneously analyzed by reversed-phase liquid chromatography-electrospray tandem mass spectrometry (RPLC-ESI-MS/MS) in the negative ionization mode, using glycocholic acid-2, 2, 4, 4-d_4 as the internal standard. The method was validated then applied to piglet bile samples from control and GLP-2 treated groups. This resulted in the identification and quantification of the 12 major BAs present in the bile. The quantitative data shows that GLP-2 does improve the clinical phenotype of PNALD by altering BA synthesis and transport. The method established here provides a powerful tool for future studies of BA metabolism in the medical and pharmaceutical fields.

19. **Detailed Characterization of the Unsaponifiable Fraction of Milk and Human Plasma Lipids by Using Enhanced Peak Capacity Chromatography and High-resolution Mass Spectrometry.** S. Salivo[1], M. Piparo[1], R. Costa*[1], P.Q. Tranchida[1], P. Dugo[1,2], and L. Mondello[1,2], [1]University of Messina, Italy, [2]University Campus Bio-Medico of Rome, Italy.

The present research is focused on the development of a comprehensive GC (GC×GC) method, with dual MS/FID detection, for the qualitative and quantitative analysis of the entire unsaponifiable fraction of milk and human lipids (plasma). The unsaponifiable fraction forms a highly specific part of lipids, and can be used not only as indicators of genuineness and quality (for food lipids), but can provide valuable information on other factors such as the instantaneous state of an organism (evaluating sterols and oxysterols). The MS result was used for qualitative scopes while the FID one was employed for quantification.

The complexity of the fingerprint, generated by the unsaponifiable fraction (no TLC fractionation has been performed), fully justified the employment of the two-dimensional GC technology. Furthermore, GC coupled with high resolution time-of-flight (HR ToF) MS was used to increase the reliability of identification of several unsaponifiable lipid constituents (particularly sterols), for which a pure standard compound was not available.

The synergism between both high-resolution chromatography and mass spectrometry processes enabled the attainment of a more-in-depth knowledge of the unsaponifiable fractions of lipid samples.

20. **Chemical Characterization of Chia (*Salvia hispanica*) Seed Oil.** M. Beccaria[1], F. Rigano[1], M. Oteri[1], G. Bartolomeo[1], V. Musarra[1], G. Tripodo[2], D. Sciarrone[1], R. Costa*[1], P. Dugo[1,2], G. Purcaro[1], and L. Mondello[1,2], [1]University of Messina, Italy, [2]University Campus Bio-Medico of Rome, Italy.

A comprehensive chemical characterization of different lipid components, namely fatty acid methyl esters (FAMEs), triacylglycerols (TAGs), phospholipids (PLs), free fatty acids (FFAs), sterols, polyphenols and tocopherols in Chia seed oil, obtained by Soxhlet extraction, was reported. Reversed phase liquid chromatography (LC) coupled to UV and mass spectrometry (MS) detectors was employed for TAGs and polyphenols determination; normal phase-LC in combination with fluorescence detector was used for tocopherols analysis; PL and FFA fractions were investigated after a rapid solid phase extraction followed by HILIC-MS and nanoLC coupled to electron ionization (EI) MS, respectively. Furthermore, FAMEs and sterols were evaluated by GC-FID and GC-MS. Results demonstrated a significant content of bioactive compounds, such as polyphenols and tocopherols, and a very high content of essential fatty acids, namely a-linolenic and linoleic acids. In addition, for the best of authors knowledge, phospholipid and FFA profiles have been elucidated for the first time. The importance of free fatty acids in food samples is due to their major bioavailability since they can be readily used in metabolic processes. For a fast and reliable determination of this chemical class, a very innovative and sensitive NanoLC-EI-MS analytical determination was applied.

21. **Determination of the Triacylglycerol Fraction in Fish Oil by Comprehensive Liquid Chromatography Techniques with the Support of Gas Chromatography and Mass Spectrometry.** R. Costa[1], M. Beccaria[1], F. Cacciola[1], M. Oteri[1], F. Franchina[1], G. Purcaro[3], P. Dugo[1,2], and L. Mondello[1,2], [1]University of Messina, Italy, [2]University Campus Bio-Medico of Rome, Italy, [3]Chromaleont s.r.l., Italy.

Fish oil made from menhaden (*Brevoortia Tyrannus*) can be used as a dietary supplement for the presence of high levels of the long-chained omega-3 fatty acids, *viz.* eicosapentanoic and docosahexanoic. Two different multidimensional approaches were developed and compared, in terms of peak capacity, for TAG characterization: silver ion chromatography and non-aqueous reverse-phase liquid chromatography were tested in both comprehensive (stop-flow) and off-line modes. The use of mass spectra attained by atmospheric pressure chemical ionization for both LC approaches, and the fatty acids methyl esters profile of menhaden oil obtained by gas chromatography analysis, greatly supported the elucidation of the triacylglycerol content in menhaden oil. On the one hand, the off-line approach afforded better separation with the possibility to identify and quantify more than 250 triacylglycerols, thanks to the higher peak capacity with respect to the stop-flow approach; on the other hand, the disadvantage of off-line analysis was the longer analysis time mainly due to manipulation and re-injection of the manually collected fractions.

22. **Improvement in MOAH Quantification in Edible Oils: Retention of Olefins by Using a Novel Liquid-liquid Gas Chromatography (LC-LC-GC) Method.** M. Zoccali[1], L. Barp[1], G. Purcaro*[2], D. Sciarrone[1], P.Q. Tranchida[1], and L. Mondello[1,2], [1]University of Messina, Italy, [2]Chromaleont s.r.l., Italy.

Mineral oil can be considered one of the most widespread food contaminants and their presence in edible oils is widely documented. Mineral oils are mainly composed of saturated hydrocarbons and aromatic hydrocarbons (MOAH). Liquid chromatography coupled to gas chromatography (GC) with flame ionization detector, represents the method of choice to determine these two families. However, despite the high selectivity of this technique, the presence of olefins, particularly squalene and its isomers, which can reach 5000 mg/kg in olive oils, does not allow the correct quantification of the MOAH fraction. In this case, additional off-line tools are needed to eliminate the olefins from the MOAH fraction. In the present research, a novel on-line LC-GC method, for the determination of hydrocarbon contamination in edible oils, is described. Two different LC columns, one of silica to retain the bulk of the matrix (fat) and one of silver-ion, which better retains the olefins, were coupled in series enabling to obtain the MOAH hump free of interfering peaks. In this way, a correct quantification of the MOAH can be achieved.

The proposed method presented good performance characteristics and it was applied to analyze different edible oils.

23. **Development of a NanoLC-EI-MS Method for the Characterization of the Free Fatty Acid Fraction in Mussels.** R. Rigano[1], A. Albergamo[1], M. Beccaria[1], S. Salivo[1], D. Sciarrone[1], G. Purcaro*[2], P.Q. Tranchida[1], and L. Mondello[1,2], [1]University of Messina, Italy, [2]Chromaleont s.r.l., Italy.

The present research is focused on the potentiality of a miniaturized liquid chromatography (NanoLC) system coupled to an electron ionization mass spectrometer (EI-MS). Such a coupling led to extend the applicability of EI-MS to typically amenable LC analytes, most of them not suitable for a gas chromatography (GC) system. The clear advantage with respect to atmospheric pressure ionization MS interface, normally coupled with LC, is the generation of a typical and reproducible fragmentation pattern, without any matrix effect or mobile phase interferences, allowing fast and reliable identification of unknowns using commercially available GC-MS libraries.

In this contribution a rapid solid-phase extraction followed by a sensitive and selective NanoLC-EI-MS analytical determination was validated and applied for the investigation of the free fatty acid (FFA) profile from lipid extracts of mussels *Mytilus galloprovincialis*. The FFAs fraction was directly injected in the NanoLC system, without any need for derivatization procedure, normally required before a GC-MS analysis. Qualitative and quantitative comparison between mussels coming from clean and polluted sites confirmed the involvement of fatty acids in important metabolic processes activated in response to environmental pollution.

24. **Reliability of the ΔECN42 Limit and Global Method for Olive Oil Purity Assessment Using Different Analytical Approach.** G. Purcaro[2,3], M. Beccaria[1], M. Oteri[1], A. Marra[1], D. Mangraviti[1], L. Conte[2], and L. Mondello[1,3], [1]University of Messina, Italy, [2]University of Udine, Italy, [3]Chromaleont s.r.l., Italy.

Two data elaboration approaches for evaluating olive oils authenticity were compared: I) the determination of the difference between the theoretical and actual amount of triacylglycerols with partition number 42 (ΔECN42 = ¦0.2¦); and II) the global method, which considers also partition number 44 and 46 (returning a "correct"/"not correct" result). Analysis of 31 genuine extra virgin olive oil samples was performed using different analytical methods, namely liquid chromatography (LC) coupled with a refractive index detector (RID) and LC coupled with a mass spectrometry detector (MS), and the results obtained were compared. Several false positives were highlighted considering the ΔECN42 limit with both instrumental approaches. Instead, the global method algorithm returned "correct" results for all the analysed samples (except for two samples which gave no results at all) with the LC-MS instrument; on the other hand 10 false positives were obtained elaborating the data deriving from the NARP-LC-RID analysis.

25. **Quantification of Brominated Vegetable Oil in Soft Drinks by Supercritical Fluid Chromatography/Mass Spectrometry.** J. Yang and J. Romano, Waters Corp., USA.

Brominated vegetable oil (BVO) is often used as a weighting agent, or a solubility-transmitter for citrus oils and other lipophilic compounds[1] in soft drinks and beverages. The US FDA has established a BVO limit at 15 ppm in finished beverages, while many countries in Europe, Asia, South America, and Australia, have banned its use in beverages. Analysis of BVO is rarely reported. Gas chromatography with mass spectrometry (GC-MS) has been proposed recently for the analysis of BVO in soft drinks and cocktail syrups[2,3]. This GC-MS method requires tedious derivatization (or saponification) of BVO, and has a long run time (about 50min).

UltraPerformance Convergence ChromatographyTM (UPC2®) is a state-of-art supercritical fluid chromatography (SFC) that provides exceptional efficiency and speed of separation4. It has been applied to a wide range of compounds, including VO, and has shown great benefits in selectivity, throughput, and ease-of-use5. This work demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC2-MS. BVO was extracted and analyzed directly without any derivatization. The chromatography total run time was 9 min. The analytical method performance (limit of quantitation or LOQ, repeatability, linearity, and recovery) as well as the analysis of BVO in soft drinks and beverages are presented.

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26. **Determination of Total Fat in Microbial Biomass by Time Domain NMR: An Alternative to FAME Analysis.** A. Shurer, A. Chang, and D. Burger, DSM Nutritional Products, USA.

In this study, we demonstrate that Time Domain Nuclear Magnetic Resonance (TD-NMR) Spectroscopy is equivalent to Fatty Acid Methyl Ester (FAME) analysis for determination of *total* fat in microbial biomass samples. TD-NMR is used extensively for determination of oil content in the seed oil industry and has demonstrated utility in microalgae as well. However, to our knowledge it is only used to quantify the *extractable* oil within a sample. We rely on measurement of the *total* lipid (including intra- and extracellular lipids, membrane lipids, etc.) in microbial biomass sampled throughout a fermentation or extraction process to guide research and development efforts toward improvements in oil production and recovery.

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. We sought to replace this method as it is not only destructive, but hazardous, time-consuming, and costly. Freeze-dried biomass samples from yeast or fungi, containing a range of fat contents (5-60%), 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 microbial biomass samples without sample preparation.

27. **A pH Responsive Hydrogel Integrated Device for Rapid Detection of Blood Triacylglycerols.** Y.S. Mugo and D. Berg, MacEwan University, Canada.

Increased levels of blood triacylglycerols (TAGs) has been linked to an increased risk of obesity and cardiovascular diseases. Maintenance of the optimum level of blood TAGs is therefore critical for the health of an individual. The presentation will demonstrate an integrated, sensitive, rapid and low cost optical biosensor with possible application for point of care detection of blood lipids. This device is based on the use of pH responsive hydrogels with high lipase enzyme loading capacity. The lipase loaded hydrogel catalyzes hydrolysis of the triacylglycerol into corresponding fatty acids, which change the pH environment of the hydrogel causing it to collapse, with this response determined by miniaturized absorbance or reflectance spectroscopy. Using triolein as a model long chain triglyceride, the device dynamic range was determined to envelope the useful TAGs clinical range of 50-5000ppm. With just a few rinsing steps, the lipase loaded hydrogel in the device could be reused for up to 3 times before significant loss of enzyme activity is observed. This talk will demonstrate the device could easily be extended to quantify compounds such as glycerol in biodiesel.

28. **Distribution of Lauric Acid Between Hexane and an Ionic Liquid.** A.W. Alkhudair[1,2], S.M. Budge[1], J.A.C. Clyburne[3], and G. Mazzanti[1,2], [1]Dalhousie University, Canada, [2]Institute for Research in Materials, Canada, [3]St. Mary's University, Canada.

Mass and molar phase diagrams of lauric acid (LA), hexane and the ionic liquid (IL) trihexyl-(tetradecyl)-phosphonium chloride were developed at room temperature. The diagrams and experimental tie-lines were obtained for the water saturated IL. The biphasic system of IL and hexane was observed for LA mole fractions less than 0.015 in IL and 0.005 in hexane. The gradual addition of LA caused the biphasic system to become monophasic at a molar fraction just below 0.01 for LA and 0.03 for IL. Composition of mixtures that phase-separated were determined by weight and gas chromatography. LA showed a solubilizing effect on the IL, since increased concentrations of the fatty acid caused an increase in the miscibility of IL with hexane. It was hypothesized that this happened due to a polar association of LA with IL at the positively charged phosphonium site. Using hexane to extract products of hydrolysis from the IL was deemed unsuitable due to the preferential distribution of LA in the IL, rather than the hexane. Phase diagrams of triglycerides in a similar system were suggested to investigate the suitability of the IL as a medium for acidolysis reactions.

29. **Skin Surface Lipid Composition Analysis of Heathy Females Utilizing Gas Chromatography-Mass Spectrometry.** J. Addy[1], T. Oliphant[1], and R. Harper[2], [1]International Flora Technologies, USA; [2]Harper & Associates, USA.

The objective of this research was to evaluate the skin surface lipid (SSL) composition of healthy 22 year old females. This information provides insight into the variation and complexity of skin lipid composition that exists within a well-defined population. The age 22 was chosen due to the fact that sebum excretion rates are at a maximum within the 16-40 year old age range (Cotterill et. al.). Subjects' foreheads were sampled for SSLs over the course of two hours utilizing lipid-free paper after a minimum of 12 hours without washing their faces. SSL composition analysis for squalene, wax esters, glycerides, free fatty acids, cholesterol, and cholesteryl esters was performed using a Gas Chromatograph-Mass Selective Detector (Agilent 6890/5973N). The data indicates that there are correlations between most of the various SSL components, with the consistent exception of the cholesteryl esters. Additionally, there was minimal variability between subjects.

30. **Bias Corrections in the Determination of Free Glycerine (FG) in B100 Biodiesel—Unexpected Formation of Glycerine Heterophases with Limited Solubility at 23-25°C.** R.W. Heiden[2] and M. Mittelbach[1], [1]University Graz, Austria, [2]R.W. Heiden Associates LLC, USA.

Residual free glycerin (FG) is a critical marker of biodiesel (B100) quality because of known deleterious effects of excessive concentrations on fuel filterability, air emissions, engine deposit

formation, and fuel storage tanks. Yet, routine determinations by internationally recognized standard methods are well known to display excessive imprecision which undermines the value of the data. We present the results of a systematic investigation of our initial observations of day to day variations in FG determinations induced by unintentional agitation. Variations caused by differential agitation between otherwise identical samples are indicative of the formation of FG heterophases with limited solubility and a potential source of sampling bias, previously unrecognized. This is a surprise because the bias is observed at the low, sub 0.02%m/m levels of FG reported in a 2011 NREL survey of commercial samples. Further, our data indicates that dissolved moisture residuals can interact with FG and cause the solubility of FG to plummet below internationally recognized limits of 0.02%m/m. Bias was averted at 0.02% FG after 3 days storage by limiting the moisture content of samples to less than 100ppm.

31. **Triacylglycerol Fingerprints by HPLC-RID and Chemometrics to Detect Extra Virgin Olive Oil Adulteration with Vegetable Oils.** A. Tres[1], S. Vichi[1], A. Pérez-Villagrasa[1], R. Codony[1], F. Guardiola[1], [1] University of Barcelona, Spain.

The high nutritional and economic value of Extra Virgin Olive Oil (EVOO) makes this product an objective of fraudulent blending with oils of different origin. The state-of-the-art strategy in food analytical authentication consists in using the raw analytical signals to find patterns (known as fingerprints) specific for the category to be authenticated, different from those in adulterated samples. The objective of this study was to develop models to detect EVOO adulteration with sunflower (SF), soybean (SY) and hazelnut oils (HZ) by measuring oil's triacylglycerol profile by a classical, relatively inexpensive and available technique (RP-HPLC-RID), using chemometrics to process data and develop the models. A total of 4 different EVOO, 4 SF, 4 SY and 4 HZ authentic oils were included in the study and blended at 0, 5 and 10% adulteration following a Latin Squares Design. Raw analytical signals were obtained and aligned. Pre-processing techniques such as 1st and 2nd derivatives or scaling were assayed. Two separate models based on Partial Least Squares Regression (PLS) were built, one for SF and SY and another one for HZ, and validated by leave 10%-out cross-validation. Models successfully detected EVOO adulteration with SF, SY and HZ.