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# Abstract

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## Virtual 2021 AOCS Annual Meeting & Expo

*Technical Program Chair: Eric Theiner, Evonik Industries, USA*

## AOCS Award Winners Series

## 2020 Alton E. Bailey Award

**Research and Development of Vegetable Oil Based Oleo-compounds as Waxes.** Tong Wang, The University of Tennessee, USA

Natural waxes of plant and insect sources have advantages over petroleum based or synthesized polymeric waxes in many industrial and food packaging applications. To mimic natural wax in both structure and functional properties, a series of vegetable oil-based waxes for coating are made and evaluated. This presentation highlights some of our work in this area. A more cohesive and softer wax compared to fully hydrogenated oil, EsterCoat, can be made from saturated fatty acids, and its application in paper coating and performance is demonstrated. A very hard and high-melting wax, AmideCoat, with the hardness of appearance as carnauba wax is made by amidation reaction with diacid, diamine, and cinnamic acid. Its application potentials are being evaluated in several industrial sectors. A high oleic soybean oil derived monohydroxy fatty acid derivative has shown to have desirable properties for fruit coating after it is formulated into a compound nanoemulsion. We have also hypothesized that to most effectively prevent moisture loss from citrus fruit, the chemical nature and crystallinity of the coating waxes, rather than their quantity play the most critical role. The 16, 22, and 26C series of hydrocarbon, alcohol, aldehyde, and acid are synthesized and their crystallinity and co-crystallization behaviors are studied. The moisture barrier property of these compounds is evaluated. These research and development examples demonstrate the possibilities of using vegetable oil-based oleo-compounds to replace petroleum and polymeric waxes for premier performance and environmental sustainability.

## 2020 Stephen S. Chang Award

**Chemical Synthesis as a Tool to Create Value in Lipid Related Products.** Dharma Kodali, University of Minnesota, USA

This presentation describes how we used chemical modifications as a tool over the years to provide value through a fundamental understanding of the structure-property-functionality of glycerol ester derivatives and in creating new products. Initially, pure stereospecific glycerol derivatives were synthesized, and the fundamental aspects of structure-property correlations were studied. Various mono-, di- and tri-acyl-sn-glycerols were synthesized by protecting and deprotecting hydroxyl function with benzyl, trityl and isopropylidene groups. The synthesis of pure partial glycerol esters presented a challenge due to acyl migration. The driving force for the acyl migration of partial glycerol esters is the efficiency of the nucleophilic attack by the adjacent hydroxyl oxygen on the carbonyl carbon leading to the formation of an isomer through an orthoester intermediate. The acyl migration was more facile in liquid form and at higher temperatures. By employing various synthetic strategies including novel reagents like bromodimethylborane and low temperatures, we were able to prevent acyl migration. Several homologous series of glycerol derivatives were synthesized and the influence of molecular packing on physical properties was studied. This fundamental understanding was then utilized to chemically modify the structure of natural oils to impart the functional properties required for new applications such as lubricants, paints and plasticizers. A number of chemical transformations such as transesterification, cyclopropanation, cyclization, epoxidation, and branching were used to generate structural heterogeneity, reduced molecular weight, disrupted packing, and a change of polarity that resulted in the required functionality and performance for a chosen application, thus creating new value-added products.

## 2020 Young Scientist Research Award

**A Nature-inspired Synthesis of Protein-phenolic Conjugates for Improved Functionalities.** Bingcan Chen, North Dakota State University, USA

Soluble bound phenolic compounds extracted from pulse deeds showed superior antioxidant activities than their free form counterparts against emulsion oxidation. Inspired by such naturally existed phenolic compounds, we synthesized novel protein-phenolic conjugates by grafting phenolics onto β-lactoglobulin (β-LG) through a mild, protein-friendly carbodiimide-mediated amide coupling reaction. The UV absorption, surface charge, and secondary structure of the formed conjugates were characterized to obtain an understanding of how the structure

of phenolics could impact the structure and property of β-LG. Electron paramagnetic resonance spectroscopy was employed to detect antioxidant activity and mechanism. The physical and oxidative stability of emulsions prepared by the conjugates was also investigated.

## 2021 Alton E. Bailey Award

**Application of Advanced Emulsion Technology in the Food Industry: A Review and Critical Evaluation.** David McClements, University of Massachusetts Amherst, USA

Emulsion technology is widely used in the food industry because many food products are emulsions, including many dressings, sauces, spreads, dips, creams, and beverages. Recently, there has been an interest in improving the healthiness, sustainability, and safety of foods in an attempt to address some of the negative effects associated with the modern food supply, such as rising chronic diseases, environmental damage, and food safety concerns. Advanced emulsion technologies can be used to address many of these concerns. In this review article, the potential utilization of these advanced technologies is critically assessed, such as nanoemulsions, Pickering emulsions, high internal phase emulsions (HIPEs), multiple emulsions, solid lipid nanoparticles (SLNs), and multilayer emulsions. In this presentation, a brief description of each type of emulsion is given, their formation and properties are described, and then their potential applications within the food industry are critically appraised.

## 2021 AOCS Award of Merit

**Stability of Edible Oils: Examining the Role of Endogenous/exogenous Antioxidants and Phenolipids.** Fereidoon Shahidi, Memorial University of Newfoundland, Canada

Oxidative stability of edible oils is dictated by their fatty acid composition and degree of unsaturation as well as their positional distribution in the triacylglycerol molecules, presence and profile as well as the concentration of minor components, and storage conditions. This study examined the role of minor components on the stability of selected oils by stripping them from the samples. While stripped oils were generally less stable than their unstripped counterparts under Schaal oven conditions, the reverse was true under fluorescent light. In addition, selected phenolipids were tested for their antioxidant efficacy. Both the structural characteristics of the phenols as well as the nature of the acyl group determined their efficacy. Finally, the phenol moieties of phenolipids were released, at least partially, upon simulated digestion and hence exerting their potential health effects.

## 2021 AOCS Fellow Award

**Sonocrystallization of Fats: Considerations for Industrial Applications.** Silvana Martini, Utah State University, USA

The elimination of partially hydrogenated oils from food formulations and the reduction of saturated fatty acids has challenged food producers to generate appropriate physical properties in healthier fats. High-intensity ultrasound (HIU) has been used over the past 15 years as an additional processing tool to improve the physical properties of fats. Studies showed that this technique is very helpful to process healthy fats that have a low level of saturated fatty acids and that crystallize slowly. Increase in crystallization rate, hardness, elasticity, and reduction in crystal size and oil migration, are some of the common physical properties that can be obtained in sonocrystallized fats. The degree of change in these physical properties is highly dependent on various processing conditions such as a chemical composition of the material, degree of agitation of the sample, heat transfer and removal of heat generated during sonication, and crystallization kinetics of the sample.

Many experiments related to the sonocrystallization of edible fats have been performed in batch proving the benefits of this technology, but only a few have shown the practical application of HIU in continuous systems similar to the ones used in industrial settings. This presentation will describe how processing conditions affect the outcomes of the sonocrystallization process. Strengths and limitations of HIU to process edible fats and the incorporation of this technology in a scraped surface heat exchanger will be discussed.

Overall, this presentation will describe important concepts that must be considered when evaluating the potential use of HIU in industrial applications.

## 2021 Schroepfer Medal

**Bile Acid Precursors: Intermediates in Cholesterol Removal or Signaling Molecules?** William Griffiths, Swansea University, United Kingdom

The bile acid "family" consists of mainly of C24 and C27 acids and C27 bile alcohols. The dominant pathway, at least in mammals, is the neutral pathway starting with 7α-hydroxylation of cholesterol by CYP7A1. However, there are numerous minor pathways which may have evolved as a route to biosynthesise bioactive intermediates, and it is these minor pathways that have been the main interest of the author over these last three decades. Mass spectrometry in combination with chromatography has greatly simplified the study of bile acids and their precursors over this time. Today, LC-MS instruments run with

minimal operator input 24/7, something that the author could only dream of in the 1980's, and provide reproducible chromatography, mass resolution in excess of 200,000 and mass accuracy of < 5 ppm, also delivering automated MS/MS and MSn, allowing identification of cholesterol metabolites at pg levels, and now even mass spectrometry imaging in tissue. Influenced by two previous recipients of Schroepfer Medal, Jan Sjövall and Ingemar Björkhem, the author has utilised LC-MS to study oxysterols, the primary cholesterol oxidation products, and their down-stream metabolism to bile acids. Following Sjövall-like methods of sample preparation and derivatisation the author and his collaborators have uncovered many unexpected cholesterol metabolites in body fluids and postulated pathways for their conversion to bile acids. Many of these intermediates have turned out to be biologically active, acting as ligands to e.g., nuclear receptors and G protein-coupled receptors. This leads to the question, have these minor bile acid biosynthesis pathways evolved to generate signalling molecules, with the ultimate formation of bile acids in the hepatocyte just providing an added bonus. In this paper, the author will expand on this hypothesis detailing methods for sterol identification, including new methods for sterol imaging in tissue, and discuss some of the unexpected pathways uncovered.

## 2021 Stephen S. Chang Award

**Stabilization of Omega-3 PUFA Rich Raw Materials and Foods Against Lipid Oxidation.** Charlotte Jacobsen, Technical University of Denmark, Denmark

Polyunsaturated long chain omega-3 fatty acids have a wide range of health beneficial effects. However, due to their polyunsaturated nature, omega-3 lipids are highly susceptible to lipid oxidation, which will result in undesirable fishy and rancid off-flavors as well as formation of unhealthy compounds.

To efficiently prevent lipid oxidation, it is necessary to understand the oxidation mechanisms and to identify the most important factors influencing lipid oxidation in each specific raw material or food system. This becomes even more important when the raw material or food system is rich in omega-3 fatty acids.

This presentation will discuss lipid oxidation mechanisms and different means to prevent lipid oxidation in various food systems such as mayonnaise and milk as well as in skin care products and in raw materials such as krill oil and side-streams from seafood processing. It will be demonstrated how oxidative stability can be improved by optimizing the processing conditions, by the use of delivery systems for omega-3 lipids or by the addition of antioxidants. The influence on lipid oxidation of the physical structure of oil-water interfaces and the location of antioxidants at the interface will also be touched upon. Examples of the effect of natural antioxidants such as seaweed extracts rich in polyphenols and peptides from potato will be given.

## 2021 Supelco AOCS Research Award

**Why Does Lipid Oxidation in Foods Continue to Be Such a Challenge?** Eric Decker, University of Massachusetts, Amherst, USA

Oxidation of lipids continues to be a challenge in many foods even after over 200 years of research. The inability to solve the problems of rancidity indicates that the process of lipid oxidation is extremely complex as it is influenced by many factors. One example is oxygen whose removal in foods that are in direct contact with oxygen is an effective antioxidant strategy while in other foods where oxygen is dissolved in the food matrix, oxygen is difficult to remove and thus is not an effective antioxidant strategy. The role of transition metals in oxidation are also complex as in some cases they have low reactivity (e.g., low moisture foods) and in others they are the major prooxidants (e.g., oil-in-water emulsions) and thus need to be the focus of antioxidant strategies. Metal reactivity is further complicated as their reactivity is dependent on their physical location, ability to be redox cycled and solubility with the latter two being influenced by other food components. The efficacy of antioxidant is also hard to predict because their activity is influenced by physical location as well as their mode of action, stability and interaction with other food components. Finally, the relationship between lipid oxidation and shelf-life is very difficult to predict which makes it difficult to develop effective antioxidant technologies. This is because lipid oxidation typically has a lag phase where off-flavors are not present followed by an exponential increase in oxidation products were sensory detection of oxidation occurs quickly. Therefore, in order to be able to predict shelf-life dictated by oxidation, more research is need on oxidation reactions during the lag phase such as loss of antioxidant and formation of early fatty acid oxidation products. By better understanding these and other factors, novel technologies can be developed to decrease food spoilage by rancidity.

## 2021 Young Scientist Research Award

**How to Use Self- and Direct Assembly to Design Smart Materials Based on Fatty Acids?** Anne-Laure Fameau, L'Oréal, France

All of the physical matter around us is composed of atomic or molecular building blocks. Controlling the assembly of these building block units holds the key for

producing materials with new properties. Our research is focused on the self- and directed assembly of matter at all length scales, i.e., from molecular to macroscopic scale. We are interested in multiscale approaches to understand the interactions, which govern the assembly of colloids both in bulk and at interfaces. We develop new strategies to control the interactions and design responsive materials. These systems could find applications in a wide range of industrial and environmental processes such as in food, cosmetics, crude oil treatment and extraction.

Soft materials, such as foams, which respond to external stimuli, are on the leading edge of materials research. The macroscopic responsivity relies on the ability to react at microscopic or mesoscopic scales. A change in the molecular structure of the surfactant activated by stimuli can affect the self-assembled structure in water and the interfacial activity, which can in turn tune the foam stability between ultrahigh stability and immediate destabilization. We will illustrate how we can use fatty acids to produce multi-stimuli responsive foams.

Other technologically important materials are made by assembling colloidal particles into structures. A variety of techniques are available to assemble particles into chains, but so far it has proven challenging to make permanent chains that are flexible. We will present a new method for making highly flexible particle chains based on capillary attractions between particles coated with liquid fatty acids, which is broadly similar to the way sandcastles are bound by small volumes of liquid. We will illustrate how the lipid capillary bridges between particles can be used to provide new opportunities for assembling particles in the form of filaments, networks and self-repairing gels.

## Analytical

*Division Vice Chair Francesca Giuffrida, Society Des Produits Nestle SA, Switzerland*

**The Insulin Centenary Song: Celebrating the Discovery of Insulin in Canada a Century Ago.** N A Michael Eskin, University of Manitoba, Canada

This year marks one hundred year since the discovery of insulin in Canada by Dr. Frederick G. Banting, an orthopedic surgeon. Dr. Banting contacted the University of Toronto and worked with Professor John MacLeod and Charles Best, a graduate student, in examining an extract from the pancreas for the treatment of diabetes, a deadly disease at that time. James Collip, a biochemist, played a crucial role in purifying the extract so it could be used as a drug. This song highlights the successful experiment performed on a diabetic dog using a pancreatic extract. This was followed by the successful treatment of a young boy dying from diabetes. As a result, many lives were saved with Banting and MacLeod both receiving the Nobel Prize in 1923, As a result, millions of diabetics around the world are able to have normal lives with continued improvements in both the insulin product and its mode of application.

## Advanced Methods of Analysis, including Lipidomics

*Chairs William Byrdwell, USDA ARS BHNRC MAFCL, USA; Arun Moorthy, National Institute of Standards and Technology*

**A Single Injection for Near-complete Structural Elucidation of Phospholipids Using Electron Activated Dissociation (EAD) in a Complex Matrix.** Mackenzie Pearson, SCIEX, USA; Takashi, Baba, SCIEX, USA; Paul Baker, SCIEX, USA; Jason Causon, SCIEX, USA

Current strategies in lipidomic analysis to fully characterize a single lipid molecular species—from sum composition to position and orientation of double bonds—generally requires several methodologies, lengthy extraction protocols, and complex separation techniques. To date, there is not a single injection solution to be able to provide a full structural snapshot of a complex lipid matrix. However, Electron Impact Excitation of Ions from Organics (EIEIO) has been recently reported on and is being utilized in an electron activated dissociation (EAD) device in a Q-TOF MS platform. Low energy electrons have been intentionally used as a dissociation tool in mass spectrometry for over a decade. Non-thermal processes by electron capture and excitation provide complemental molecular structure information in addition to conventional collisional activation. An issue with electron-based techniques is the poor interaction efficiency between electrons and ions. We developed a new type of radio-frequency ion trap that allowed simultaneous injection of ions and electrons to improve efficiency.

Using EAD with an information dependent acquisition (IDA) experiment, we are able to identify and fully characterize lipid species in bovine liver extract. By also employing a time-scheduled MRMHR experiment with CID fragmentation and EAD fragmentation concurrently, we were able to compare MS/MS spectra for single lipid species. Limited information was observed in the CID experiment making complete structural analysis on a lipid molecular species challenging or simply not achievable. With EAD fragmentation, a lipid's class, acyl chain regioisomerism, and double bond number and location can be clearly observed. Overall, the EAD device coupled with a Q-TOF-MS system is especially powerful for lipid analysis, due to its high efficiency of fragmentation and high sensitivity of the TOF-MS.

**Beyond 2D-LC: Three Dimensions of Chromatography with Quadruple Parallel Mass Spectrometry, LC3MS4, for Infant/adult Formula Analysis.** William Byrdwell, USDA ARS BHNRC MAFCL, USA

Two-dimensional liquid chromatography (2D-LC) is becoming less exotic and more mainstream as commercially available instruments proliferate. Despite the impressive capabilities of 2D-LC, there are times when even 2D-LC is not sufficient for an adequate separation. One-dimensional reversed-phase HPLC is not adequate to completely resolve all milk (or infant formula) triacylglycerol (TAG) molecular species, which are highly complex, and contain many isomers of TAGs, especially TAGs with short-chain fatty acids. Even conventional 2D-LC is not completely satisfactory. A new approach was developed, in which two second dimensions were used simultaneously for dual parallel comprehensive 2D-LC with quadruple parallel (x4) mass spectrometry. A pair of contact-closure (CC) controlled UHPLC switching valves were coupled to a timed CC circuit to allow the second 2D separation, 2D(2). The first second dimension, 2D(1), used the commercially available system, in which conventional 2D-LC improved the separation of components, and all components eluted in the first modulation period from a 50 mm C30 column. The 2D(2), which employed a 100 mm C30 column, produced an even better separation of components, but required more than one modulation period to elute, resulting in multi-cycle chromatography or ''controlled wraparound'' chromatography, analogous to twin column recycling chromatography. Electrospray ionization (ESI) mass spectrometry (MS) was used in parallel with atmospheric pressure photoionization (APPI) MS for LC1MS2 to monitor the first dimension RP-HPLC, while ESI-MS monitored the 50 mm C30 UHPLC (LC1MS1) and ESI-MS also monitored UHPLC using the 100 mm C30 column (LC1MS1), to produce LC1MS2 x (LC1MS1 + LC1MS1) = LC3MS4.

**Improving Workflow of the Ambient Ionization Mass Spectrometry Lipid Analysis by Utilizing a Pulse Ionizing Gas Enabled Direct Analysis in Real Time (DART) Source.** Brian Musselman, IonSense, Inc., USA; Scott Campbell, Spectral Works, United Kingdom; Frederick Li, IonSense, Inc. USA

The utility of direct analysis in real time (DART) for thermal desorption and ambient ionization mass spectrometry of lipids has been recognized since inception of the method in 2005. Implementation of schemes to facilitate automated analysis 10 years ago served to increase the throughput of the method. Numerous publications have demonstrated the utility of statistical analysis to permit rapid comparison of samples in minutes. However, notable complications to the use of ambient ionization such as the observation of ionized contaminant derived from the atmosphere and difficulties in peak detection can impact quality of the data. A standard DART ionization source controller has been modified to deliver a short pulse of ionizing gas coordinated with the movement of sample into the sampling region. The gas temperature and time of pulse duration are set to optimize the generation of sample related ions while minimizing the generation of ions from gases present in the ambient atmosphere. Timing of movement of the next sample into position is varied to reduce the potential for cross contamination thus permitting the use of higher density sample arrays including 96-well and 384-well format when analyzing large sample sets. Examination of the utility of this pulsed gas method for rapid determination of lipid profiles using different temperatures and ion polarities enables more accurate lipid profiling utilizing statistical analysis. The discontinuous application of ionizing gas offers the benefit of less gas consumption and facilitates automated peak detection as neither sample nor background-related ions are generated when the ionizing gas is turned off.

Application of the new method for rapid lipid profiling and detection of lipid quality, type, and presence of contaminants will be presented.

**Lipid Separation and Structural Characterization Using ACQUITY PREMIER Technology and Travelling Wave Ion Mobility.** Giorgis Isaac, Waters Corporation, USA; Nyasha, Munjoma, Waters Corporation, United Kingdom; Martin Palmer, Waters Corporation, United Kingdom; Lee Gethings, Waters Corporation, United Kingdom; Robert Plumb, Waters Corporation, USA

Lipids represent a diverse group of biomolecules that have an essential role in structural, storage and signaling processes in living systems. The analysis and structural characterization of lipids remain challenging due to the chemical structure diversity and isobaric nature. The main objective of this presentation is to add ion mobility (IM) and recent PREMIER LC technology to the lipidomic workflows to enhance separation and structural characterization of lipids.

The data for the comprehensive lipidomics database was collected on a SYNAPT-XS and for lipid structural characterization on a hybrid quadrupole cyclic IM orthogonal acceleration time-of-flight (Q-cIM-oaToF) instrument. Ion mobility separation is achieved using a single and multi-pass travelling-wave cyclic IM (cIM). MS and CID fragmentation data were obtained on precursor IM separated lipids for structural characterization of lipids.

Unknown analytes/features represent a bottleneck in untargeted lipidomics research. Ion mobility-mass spectrometry (IM-MS) facilitates lipid identification because it yields collision cross section (CCS) information that is independent from mass or lipophilicity. A comprehensive CCS lipidomics database was developed that contains accurate mass (m/z), retention time (RT), fragment ion and CCS values and applied to a biological lipidomics data set. CCS provides additional

separation dimension that allows the separation of isobaric and isomeric compounds. This provides confidence in lipid identification. The separation and structural characterization of different lipid classes using PREMIER technology and cIM is currently under study. The potential of ion mobility for the separation of positional isomers and structural characterization including double bond localization will also be investigated.

**Simple, Rapid Lipidomic Analysis of Triacylglycerols in Bovine Milk by Infusion-electrospray mass Spectrometry.** Michael Bukowski, USDA-ARS, USA; Matthew Picklo, USDA-ARS, USA

Bovine milk is a complex mixture of lipids, proteins, carbohydrates and other factors of which lipids comprise 3–5% of the total mass. Rapid analysis and characterization of the triacylglycerols (TAG) that comprise about 95% of the total lipid is daunting given the numerous TAG species. In this work, we demonstrate an improved method for identifying and quantifying TAG species by infusion-based "shotgun" lipidomics. Because of the broad range of TAG species in milk, a single internal standard was insufficient for the analysis and required sectioning the spectrum into three portions based upon mass range to provide accurate quantitation of TAG species. Isobaric phospholipid interferences were removed using a simple dispersive solid-phase extraction step. Using this method, > 100 TAG species were quantitated by acyl carbon number and desaturation level in a sample of commercially purchased bovine milk. Updated processing methods will also be presented for automated sample preparation.

**The Skinny on Fats—an Overview of Advanced Analytical Techniques for Lipid Analysis.** Eberhardt Kuhn, Shimadzu Scientific Instruments, USA

Lipids are a broad class of compounds that are essential to life. They include fatty acids, triglycerides, phospholipids, and many more. Because they are structurally and functionally diverse, their analysis can be challenging. This presentation will give an overview of the latest advanced analytical techniques and methods for the analysis of fatty acids and triglycerides, such as GCMS, LCMS/MS, SFCMS/MS, and MALDI-TOF-MS. Specific methods presented here include:

A novel technology for determination of the position of double bonds in unsaturated fatty acids. Solvent Mediated Chemical Ionization (SMCI) enables unambiguous assignment of chemical structures to fatty acids (FAMEs) without chemical standards, which are not readily available for most FAMEs.

A fast LCMS/MS method for triglycerides that allows the screening of around 150 Triacylglycerols in 10 minutes in human serum as well as food samples.

An LCMS method for high-throughput monitoring of fatty acid metabolites in human plasma. This method can monitor 114 fatty acid metabolites and quantitatively detect over 30 fatty acid metabolites including eicosanoids, DHA metabolites and linoleic acid metabolites in human plasma in 10 minutes.

An SFC/MS method for triglycerides that also discusses the influence of stationary phase chemistry of four different columns on the analysis.

A simple and rapid detection method for lipid degradation products induced by heating olive oil. This method compares triacylglycerols (TAG) and TAG oxides before and after heating using a benchtop MALDI-TOF MS.

## Analysis of Less Abundant Lipids

*Chair Kim Ekroos, Lipidomics Consulting Ltd., Finland*

**Destruction of Polyunsaturated Alkyl and Alkenyl Gropcho of Plasma Lipoproteins During Incubation with Groups V and X Secretory pla2s.** Arnis Kuksis, University of Toronto, Canada

Previous studies have demonstrated that alkyl and alkenyl GroPCho require Ca++ independent PLA2 for hydrolysis and are not hydrolyzed by Ca++ dependent sPLA2s. We report extensive degradation of alkenyl (arachidonoyl) GroPCho during prolonged incubation (sPLA2s, 37 °C, 0–24 hours). Diacyl GroPCho depleted alkyl and alkenyl GroPCho samples were prepared by hydrolysis with group V or X sPLA2s, which retained the diradyl GroPCho in digestion residue. Continued incubation resulted in gradual disappearance of the diradyl GroPCho peak in the LC/ESI/MS total ion current profile and in appearance of new peaks in the lysoPtdCho elution range. Non-enzymatic destruction of alkenyl GroPCho had been previously demonstrated in the presence of a free radical initiator (AAPH) (Khaselev and Murphy, 2000). Our incubates were examined for the presence of the previously identified degradation products. We located the OH/20:4- and 1:0a/20:4- and 16:0p/5:0c-GroPCho as major products (0.5–2.5 nanomoles/ mg protein) and other minor derivatives in LDL, HDL and HDL3. None of these derivatives of plasmalogens have been shown to occur in plasma lipoproteins. We speculate that the loss of alkyl GroPCho, which has no vinyl ether bond, may be due to hydrolysis by endogenous PAF acetylhydrolase following shortening of the arachidonate chain.

**Development of an On-tissue Derivatization Method for MALDI Mass Spectrometry Imaging of Bioactive Lipids Containing Phosphate Monoester Using Phos-tag.** Kuniyuki Kano, The University of Tokyo, Japan; Taiga Iwama, Tohoku University, Japan; Daisuke Saigusa, Tohoku Medical Megabank Organization, Japan; Kim Ekroos, Lipidomics Consulting Ltd., Finland; Junken Aoki, The University of Tokyo; Japan

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is an emerging label-free method for mapping the distribution of diverse molecular species, including lipids in tissue sections. However, imaging of minor bioactive lipids, lysophosphatidic acid (LPA) and sphingosine-1-phosphate, has been challenging due to their low abundance. To overcome the problem, we have developed a novel on-tissue derivatization method using Phos-tag, a zinc complex that specifically binds to a phosphate monoester group, which is a functional residue common to LPA and S1P. Using the optimum condition, MALDI-MSI with Phos-tag derivatization successfully permitted visualization of LPA and S1P in the murine brain with high sensitivity and spatial resolution (10 μm). Moreover, the Phos-tag derivatization allows for simultaneous mapping of lipids with phosphate-monoester, including phosphatidic acid and ceramide-1-phosphate. When compared with conventional MALDI-MS, this derivatization produced clear LPA images discriminating LPA artificially produced during MALDI-MS analysis. In mice with deficiencies in enzymes that degrade LPA and S1P, we observed marked S1P and/or LPA accumulation in specific regions of the brain. Therefore, this derivatization method is an innovative way to reveal the spatial localization of LPA and S1P in biological tissues.

**Evaluation of Supercritical Fluid Chromatography Coupled with High Resolution Mass Spectrometry for the Analysis of Species of Minor Lipid Classes.** Francesca Giuffrida, Nestle Research Ctr, Switzerland; Isabelle Tavazzi, Societes des Produits Nestlé, Switzerland; Francesco Contin, Bologna University, Italy; Kim Ekroos, Lipidomics Consulting Ltd., Finland

The analysis of lipid species belonging to minor lipid classes and sub-classes such as lysophospholipids, glycosphingolipids, mono- and diacylglycerols and sterols, is challenging due to their low abundance and different affinity to polar and apolar solvents.

Reverse-phase and Hilic-based liquid chromatography coupled with mass spectrometry are usually used to identify minor lipid species and classes. However, these technologies do not permit to analyse a broad range of lipids due to their insufficient polarity ranges. In parallel, they require the use of relatively large amount of solvent.

In this study, supercritical fluid chromatography coupled with high resolution mass spectrometry (SFC-HRMS) was evaluated as a green and holistic technology for the identification of minor lipid species and classes in different matrices.

Species of minor lipid classes, i.e., sterol esters, mono- and diacylglycerols, free fatty acids, and ceramides were well separated and detected under the same SFC conditions, showing the feasibility of using SFC to monitor minor lipid species characterized by different polarity.

**GC-MS Analysis of Very Long Chain Fatty Acids in Human Plasma**. Heather Kuiper, CDC, USA; Cynthia Campbell, CDC, USA; Hubert Vesper, CDC, USA

Very long chain fatty acids (VLCFA) are fatty acids with carbon chains longer than C22. VLCFA are associated with diseases such as metabolic syndrome and macular degeneration; however, the abundance of VLCFA in blood is typically below 1% of total FA. In order to better understand the role of VLCFA in human health and disease, a sensitive and specific measurement approach is needed. The CDC measures FA in human plasma using isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry (ID-GC-NCI-MS) of FA pentafluorobenzyl-esters. Our method is a sensitive and selective approach that can be beneficial for low abundance FA. We applied our method to the analysis of VLCFA in human plasma.

Using ID-GC-NCI-MS in conjunction with PFB-Br derivatization, the PFB-esters are cleaved in the ion source of the mass spectrometer. This approach produces a single intact carboxylate anion instead of the many fragment ions typically observed in EI. Analyzing FA with NCI and PFB-Br derivatization resulted in a twentyfold increase in sensitivity over EI analysis of FAMEs. Our laboratory method allowed us to detect VLCFA, but the analytical run time was quite long since we were using a 200 m Select-FAME column. Therefore, the existing method was modified by using a shorter GC column (Phenomenex 30 m ZB-5MSi column). This reduced the analysis time for VLCFA from 140 min to 20 min. With this modified method, we screened 73 human plasma samples for C24:4, C24:5, C24:6, and C26:6. We detected all four VLCFA in at least 95% of the samples. These VLCFA were present in low nanomolar concentrations in plasma, requiring very specific and sensitive analytical approaches. The m/z specificity obtained with PFB-esters and single ion monitoring, along with the sensitivity of analyzing intact carboxylate anions allow for the analysis of low abundance VLCFA in human plasma.

**Mapping Dynamic Lipid Biochemistry at Cellular Resolution Using Advanced Mass Spectrometry Imaging Technologies.** Shane Ellis, University of Wollongong, Australia

Mass spectrometry has evolved into an indispensable tool in the (bio)chemical sciences. However, analyses are typically performed on extracted molecules from homogenised samples meaning the spatial context in which molecules are present is sacrificed. Understanding not only what molecules are present, but where there are located within a tissue or a cell and how they are altered during the progression of disease is of utmost importance for understanding complex biochemical processes occurring in spatially heterogeneous tissues. In this presentation, I will demonstrate how recent advances in mass spectrometry imaging (MSI) now provide a suite of potent approaches to

study chemical diversity direct from complex surfaces like biological tissues. Unlike other imaging approaches, MSI allows the parallel imaging of thousands of molecules without the need for labels and with cellular-level resolution.

I will give an introduction to matrix-assisted laser desorption/ionisation-MSI (MALDI-MSI) and its applications for visualising biochemical processes within tissues. Particular emphasis will be placed on innovative technology developments made by my group that enable enhanced chemical resolution and detection of previously undetectable molecular classes. These include the development of MALDI-MSI ion sources for Orbitrap mass spectrometers, highly parallelised MS/MS imaging acquisitions sequences, laser-induced post-ionisation methods that increase sensitivity 100-fold and the imaging of isomeric lipids using selective gas-phase ion/molecule reactions. The coupling of MSI with high resolving power analysers also enables localised molecular dynamics to be visualised in tissues, and we have applied this in mouse models of respiratory disease. These advances pave the way of dynamic MSI where lipid turnover rates are provided concurrently with imaging data, thus providing a new window into tissue heterogeneity.

**Using Reference Materials to Generate Biological Reference Ranges in Lipidomics.** Federico Torta, National University of Singapore, Singapore

For a lipid to be useful in the clinic, physicians must know its levels in a healthy person. Lipidomics researchers are working toward filling this gap through a collegial effort. In April 2017, members of several research groups specialized in various lipid chemistries met in Singapore with a mission: figure out how to harmonize studies of the human plasma lipidome. The group settled on ceramides as the first class of molecules to be measured, as they have roles in disease and are thought to be abundant, stable and easy to isolate. A ring trial was launched, distributing the NIST SRM 1950 plasma, and 3 other new reference materials, to about 30 participant labs around the world. The goal was to measure the absolute concentration of four selected ceramides. This exercise allowed us to learn more about inter-assay and inter-lab differences, how data analysis might impact final results and it extends the findings obtained from previous similar initiatives.

More trials are following, with the aim of measuring absolute concentrations of other lipid classes in reference materials. After this first stage, phase two of the project will involve collecting samples from different human cohorts and measure the same lipids, generating reference ranges in healthy and disease conditions, driving a translation towards the clinical use of lipidomics.

## Analytical Division Student ePoster Pitch Competition

*Judges Lisa Clement, Cargill Inc., USA; Sneh Bhandari, Consultant, USA; Arun Moorthy, National Institute of Standards and Technology, USA*

**A rapid and efficient method for dialkyl ketones and sterols determination in fat.** Steven Mascrez, Gembloux Agro Bio Tech, University of Liège, Belgium; Sabine Danthine, University of Liège, Belgium; Giorgia Purcaro, University of Liège, Belgium

The present work deals with the optimization of a fast method for the determination of dialkyl ketones (DAKs) in interesterified fats. Microwave-assisted saponification was optimized, followed by purification on a lab-packed silica solid-phase extraction cartridge. The method proposed may be used for the determination of DAKs or both DAKs and sterols by simply eluting an additional fraction from the solid-phase cartridge. The final determination was performed by gas chromatography-flame ionization detector (GC-FID) for quantification and gas chromatography-mass spectrometry (GC-MS) for confirmation purposes. The proposed method showed good recoveries ( >80%) and limit of quantification (0.04–0.07 μg/g for the 4 standard DAKs and of 0.07 μg/g for α-cholestanol). Repeatabilities (n=3) were below 15% for DAKs and generally lower than 6% for sterols. Accuracy on the entire sterol profile was confirmed in comparison to the International Olive Council reference method. The method was finally applied to real-world samples before and after chemical interesterification.

**Extraction of dairy phospholipids using switchable solvents.** Kaavya Rathnakumar, MEngg, South Dakota State University, USA; Sergio Martinez-Monteagudo, New Mexico State University, USA

Milk phospholipids (PLs) are recognized for their health benefits. Currently, extraction of the PLs fraction from concentrated streams results in low efficiency, and it involves subsequent solvent separation and lipid recovery. This research aims at developing a PLs extraction process from dairy byproducts using switchable solvents. The new generation of solvents are smart and can be reversibly switched between a hydrophobic and hydrophilic form by simply bubbling or removing CO2 and can be reused. This novel approach requires neither distillation nor the use of volatile, flammable or chlorinated solvents. Different dairy by-products streams were used to evaluate the feasibility of switchable solvent extraction. A tertiary anime (N, N-dimethyl cyclohexylamine, CyNMe2) was used as a switchable solvent. The recovered PLs were quantified by thin-layer chromatography and HPLC-CAD. For comparison, PLs were also extracted using the Folch method (FM) and the Mojonnier method (MM). Statistical analysis was carried out to identify the significance of different process parameters. The extraction

efficiency of CyNMe2 ranged from 0.33–99%, depending on the type of byproduct. Remarkably, the CyNMe2 extracted up to 99% of the PLs directly from buttermilk. Improvements in the extraction efficiency of PLs from B-serum was done by the effect of acoustic intensity (44.56 $\pm$ 3.47 W cm-2) before CyNMe2 extraction (1/12mL) ratio recovered 69.07 $\pm$ 0.11% of PLs, a 9.8-fold increment than the samples without ultrasound-pretreatment. The recovered fraction of PLs mainly comprised of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%). Optimization was done with maximum desirability of 0.982 and the model was significant. The model was significant for all three responses and the co-efficient of determination(R2) were 0.9825, 0.8164 and 0.7060 for 3, 10, 18 h. For CyNMe2, scanning and confocal microscopy-images, particle size, and gel electrophoresis revealed great disruption of the protein matrix, releasing the PLs into the aqueous medium.

**Multiple-cumulative trapping non-saturated headspace SPME: A powerful tool to increase volatile fingerprints.** Steven Mascrez, Gembloux Agro Bio Tech, University of Liège, Belgium; Giorgia Purcaro, University of Liège, Belgium

Headspace solid-phase microextraction (HS-SPME) is a solvent-free sample preparation method widely applied in many fields of applications. The theory that underlays this extraction technique is based on the equilibrium among a three-phase system, i.e., sample-headspace-fiber. A compromise between sensitivity and extraction time is usually needed to optimize the sample throughput, mainly when a large number of samples are analyzed, as usually the case in cross-samples studies. This work explores the capability of multiple-cumulative trapping solid-phase microextraction on the characterization of the aroma profiling of olive oils, exploiting the automation capability of a novel headspace autosampler. It was shown that multiple-cumulative solid-phase microextraction has the potential to improve the overall sensitivity and burst the level of information for cross-sample studies by using cumulative shorter extraction times. Moreover, contrary to what is usually applied, the selection of the sample volume which does not saturate the headspace (i.e., even 10 times lower than the commonly used quantity), is of high relevance in order to maximize the information extractable.

In this work, the fingerprinting of olive oil is explored to discriminate among extra virgin, virgin, and lampante oil, a challenging task of interest for quality and authenticity assessment. The different conditions were compared, considering the general information as pattern analysis, rather than intensity-wise. The best results were obtained by using 0.1 g of samples, rather than the typical 1.5 g (or even more) found in the literature. Moreover, the use of multiple-cumulative extraction allows for better discrimination among the different groups compared to a single extraction. Finally, the increased separation power obtained by two-dimensional comprehensive gas chromatography (GC$\times$GC) was exploited to enhance further the identification capability.

## AOCS Official Methods

*Chair Susan Seegers, MS, Bunge*

**New AOCS Methods and Methods Under Development.** Scott Bloomer, American Oil Chemists' Society, USA

Recently revised AOCS methods will be reviewed. An overview of a substantial revision and scope expansion of the AOCS method for measuring trypsin inhibitor activity will be presented. AOCS Procedure M 2–09, Writing and Approval of Methods was substantially revised, and three templates were developed to simplify the process of developing new AOCS methods. Ongoing method revisions include Ce 6–86, HPLC analysis of phenolic antioxidants; and Ce 5–86, Triglycerides by Gas Chromatography. A method for analysis of tocopherols and sterols in deodorizer distillates and other related matrices by gas chromatography is also under development.

## Authentication of High Value Oils, Including Olive Oil.

*Chairs Selina Wang, University of California, Davis, USA; Luisito Cercaci, Pompeian Inc., USA*

**A Peer Inter-laboratory Study of SPME-GC-FID/MS Method for the Analysis of Volatile Compounds in Virgin Olive Oils for Supporting Panel Test: Extracted Conclusions and Future Directions.** Diego Luis Garcia Gonzalez, Instituto de la Grasa (CSIC), Spain; Enrico Casadei, Alma Mater Studiorum - Università di Bologna, Italy; Enrico Valli, Alma Mater Studiorum - Università di Bologna, Italy; Ramón Aparicio-Ruiz, Instituto de la Grasa (CSIC), Spain; Clemente Ortiz Romero, Instituto de la Grasa (CSIC), Spain; Maurizio Servili, Università degli Studi di Perugia, Italy; Roberto Selvaggini, Università degli Studi di Perugia, Italy; Florence Lacoste, Institut des Corps Gras, France; Julien Escobessa, Institut des Corps Gras, France; Stefania Vichi, Universitat de Barcelona, Spain; Beatriz Quintanilla-Casas, Universitat de Barcelona, Spain; Pierre-Alain Golay, Nestle Research, Switzerland; Paolo Lucci, Università degli Studi di Udine, Italy; Erica Moret, Università degli Studi di Udine, Italy; Alessandra Bendini, Alma Mater Studiorum – Università di Bologna, Italy

The sensory assessment of virgin olive oils is officially carried out by a sensory panel that identifies positive

and negative sensory attributes to classify the oils according to their quality grades, namely extra virgin (EV), virgin (V) and lampante (L). The workload of sensory panels, the limited number of them and the need for a confirmative robust classification, particularly in borderline oils (EV vs. V, V vs. L), has led to the search of instrumental methods supporting the sensory assessment. In this context, the identification and quantification of volatile compounds responsible for sensory attributes of virgin olive oil by solid phase microextraction-gas chromatography-flame ionization detector/mass spectrometry (SPME-GC–FID/MS) has been tested in an inter-laboratory approach analyzing the same samples, identifying the same compounds, and implementing the same quantification procedure. In this work, 7 laboratories participated and the information provided by them were differentiated according to the detector that they used, FID or MS. Linearity, repeatability, reproducibility, recovery and limits of detection and quantification were studied. Additionally, the laboratories tested three alternative strategies of quantification, all based on calibration curves, and the results were compared. The limits of detection and quantification were also studied by means of more than one method to analyze the most representative ways to measure the signal to noise ratio at low concentration. The discussion allowed implementing some improvements of the protocol towards a full validation process. This work was developed in the context of the project OLEUM ‘‘Advanced solutions for assuring authenticity and quality of olive oil at global scale funded by the European Commission within the Horizon 2020 Programme (2014–2020, grant agreement no. 635690).

**Avocado Oil — Factors Influencing Composition and Quality.** Marie Wong, Massey University, New Zealand; Cecilia Requejo-Jackman, The New Zealand Institute for Plant and Food Research Limited, New Zealand; Laurence Eyres, ECG Consulting Ltd, New Zealand; Allan Woolf, The New Zealand Institute for Plant and Food Research Limited, New Zealand

Extraction of avocado oil for use as a culinary oil has grown in popularity around the world along with a growth in plantings of avocado orchards in sub-tropical regions. The primary cultivar grown worldwide is the ‘Hass’ cultivar with also significant plantings of other cultivars such as ‘Fuerte’, and ‘Pinkerton’. Avocado oil is a healthy plant oil with a fatty acid composition high in monosaturated fatty acids, high concentrations of phytosterols and antioxidants (tocopherols and carotenoids). The composition of avocado oil is influenced by the cultivar, region of growth and climate, pre- and post-harvest factors and processing conditions. Small variations in the fatty acid profile have been found in avocado oil from different cultivars and from different regions. Fruit maturity can influence fatty acid composition while fruit ripeness can influence antioxidant composition and oil quality. Research completed in New Zealand on factors influencing the composition and quality of avocado oil will be reviewed in this presentation.

**Large-scale Evaluation of Shotgun Triacylglycerol Profiling for the Fast Detection of Olive Oil Adulteration.** Beatriz Quintanilla-Casas, Universitat de Barcelona, Spain; Giulia Strocchi, Universitat de Barcelona, Spain; Julen Bustamante, Universitat de Barcelona, Spain; Berta Torres-Cobosa, Universitat de Barcelona, Spain; Francesc Guardiola, Universitat de Barcelona, Spain; Wenceslao Moreda, Instituto de la Grasa (CSIC), Spain; José Manuel Martínez-Rivas, Instituto de la Grasa (CSIC), Spain; Enrico Valli, Alma Mater Studiorum - Università di Bologna, Italy; Alessandra Bendini, Alma Mater Studiorum - Università di Bologna, Italy; Tullia Gallina Toschi, Alma Mater Studiorum - Università di Bologna, Italy; Alba Tres, Universitat de Barcelona, Spain; Stefania Vichi, Universitat de Barcelona, Spain

According to the last annual report of the EU food fraud network, olive oil (OO) became the most notified food product in terms of non-compliances. In this context, the blending with oils of lower economic value has been reported to be among the most common economically motivated adulterations in OO. This fact reveals the need for fast and high throughput methods that allow an efficient wide-ranging screening analysis in the detection of OO illegal blends, even at low levels of adulterants with a similar fatty acid composition to OO.

Hence, the present study aims to develop authentication models for the comprehensive detection of OO illegal blends, whose adulterant included different types of high linoleic (HL) and high oleic (HO) vegetable oils at low concentration rates (2–10%). The proposed experimental design also considered the actual variability of genuine OOs by including oils from two harvest-years, several geographical regions and olive cultivars.

The final set, consisting of more than thousand samples, was analysed by Flow Injection Analysis-Heated Electrospray-HRMS (FIA-HESI-HRMS) in order to obtain a detailed triacylglycerol (TAG) profile, including both major and minor compounds. A combined PLS-DA binary modelling based on shotgun TAG profiling proved to be a fit for purpose screening tool in terms of efficiency and applicability. The external validation resulted in the correct classification of the 86.8% of the adulterated samples (diagnostic sensitivity=0.87), and the 81.1% of the genuine samples (diagnostic specificity=0.81), with an 85.1% overall correct classification (efficiency=0.85).

**Metabolic profile obtained by 1H NMR spectroscopy explains the high dependence of International EVOO blends characteristics from Italian oil content.** Francesca Caló, University of Salento, Italy;

Chiara Roberta Girelli, University of Salento, Italy; Federica Angilé, University of Salento, Italy; Laura Del Coco, University of Salento, Italy; Lucia Mazzi, Certified Origins, Italy; Daniele Barbini, Certified Origins, Italy; Francesco Paolo Fanizzi, University of Salento, Italy

The authenticity of a high-value product such as extra virgin olive oil (EVOO) is guaranteed by monitoring the geographical origin, as well as quality parameters. This is particularly important in a continuously evolving global context, which witness new producers engaged in EVOO blends constitution. For this reason, the present work aims to study the influence of each oil component on the overall characteristics of international blends. More oils from geographical areas within and outside the European community (CE) were examined using 1H NMR spectroscopy associated with chemometric methods. Looking at statistical models (unsupervised PCA and supervised PLS-DA/OPLS-DA) obtained from NMR spectra data, a clear dependence of the blend EVOOs characteristics on the increasing content of Italian rather than the Tunisian, Portuguese, Spanish or Greek oils emerged. In particular, a linear progression of the metabolic profile defined characteristics for the analysed samples — up to a plateau level — was found in relation to the content of the main constituent of the Italian oil. The Italian constituent percentage appears to be correlated with the ratio of unsaturated/saturated fatty acids and the polyphenols content as major and minor components respectively. The obtained results confirm the efficiency of the NMR based statistical protocol as a powerful scientific method for product characterization and traceability.

**Overview of Frauds in Virgin Olive Oils and New Markers and Analytical Methods to Detect Their Authenticity and Quality: The OLEUM Project.** Tullia Gallina Toschi, Alma Mater Studiorum - Università di Bologna, Italy

Extra virgin olive oil (EVOO) is recognized worldwide for its unique characteristics which include its sensory attributes, the physic-mechanical processes for its production and its reputation as one of the healthiest sources of dietary fats and minor compounds (e.g., polyphenols). These characteristics, among others, make EVOO a cornerstone of the Mediterranean diet and a food with high commercial value and attractive for consumers, but at the same time remains one of the most highly targeted by fraudsters, on the market. In this presentation the results obtained from international on-line surveys conducted by involving EU and non-EU stakeholders in the olive oil sector in the framework of the Horizon 2020 EU OLEUM project will be discussed. Furthermore, from the elaboration of the questionnaires on the common and emerging fraud issues addressed to the EU Food Fraud Network (FFN) National Contact Points, it will be highlighted that some of the respondents indicated specific fraudulent practices related to the olive oil that are occurring also because the lack of appropriate analytical methods. These problems, as well as some possible or specific analytical solutions proposed by the respondents and the OLEUM methods developers, will be critically presented.

This work is developed in the context of the project OLEUM ‘‘Advanced solutions for assuring authenticity and quality of olive oil at global scale’’, funded by the European Commission within the Horizon 2020 Programme (2014–2020, GA no. 635690). The information expressed in this abstract reflects the authors’ views; the EC is not liable for the information contained therein.

**Understanding How Common Adulterants and Natural Factors Impact the Chemical Composition of Avocado Oil**. Hilary Green, University of California, Davis, USA; Selina Wang, University of California, Davis, USA

The avocado oil industry continues to grow and with it there is an increasing demand for research contributing to the standard development effort. Last year we discovered that many avocado oils on the market were either adulterated with cheaper oils or were of poor quality. There were also many oils that had purity values outside the range of current proposed standards and literature values, though not significant enough to confirm an economic adulteration. Considering this, it is imperative to understand how both common adulterants and natural factors impact the chemical composition of avocado oils so that natural variation can be distinguished from adulterations. Here, we deeply investigate common purity parameters, including fatty acid trans and cis isomers, in market avocado oils and samples made in lab. The pure lab-made oils account for a variety of factors that contribute to natural variance, including two cultivars, Hass and Carmen, harvest times throughout the season, and processing variables. The chemical composition of known adulterated oils samples were compared to the pure lab-made samples to determine if there were any compounds that could be used as markers for adulteration. One oleic fatty acid isomer shows promise in helping to differentiate pure avocado oil from common adulterant oils, which could be utilized by industry in the future. This work will also help contribute to standard development to ensure purity standards accommodate natural variance, at the same time, limit adulterations.

## Dutton Award Symposium: Toward Complete Characterization of Fatty Acid Structure

*Chairs Pierluigi Delmonte, US Food and Drug Administration, USA; Tom Brenna, University of Texas at Austin, USA*

**Deducing Structures of Unusual Fatty Acid Methyl Esters with High Sensitivity Without Chemical Standards.** Tom Brenna, University of Texas at Austin, USA

The vast quantity of fatty acids produced and consumed have straight chains, and double bonds are of cis (Z) geometry, and when polyunsaturated, are methylene-interrupted. FAME have superb analytical properties on high resolution capillary GC columns.

Mass spectrometry (MS) is the most sensitive method for fatty acid analysis. Electron ionization (EI) of FAME causes rearrangement of double bonds and is unsuitable for deducing unknown structures. Specialized esters provide structural detail, however methods to characterize FAME directly are preferred. The revolution in MS techniques has resulted in refinement of physical methods, particularly high mass resolution and exact mass measurement, that excel at characterizing ions that include heteroatoms, however they do not distinguish isomers. That problem remains one requiring fragmentation possibly after selective derivatization, both fundamentally chemical processes.

We developed a gas phase ion-molecule reaction termed covalent adduct chemical ionization (CACI) which results in derivatives that, upon collisional activation, yield fragments that identify double bond structure and in some cases, geometry. Separately, we realized that collisional dissociation of EI generated molecular ions yields very different mass spectra than MS-1 with, for instance, no McLafferty rearrangement ion. Instead, strong ions characteristic of chain branching in saturated FAME are observed. Recent advances in these methods are extension to modern GC/MS/MS instruments via solvent-mediated (SM) CI on Shimadzu instruments, as well as combining the power of these approaches using low energy CID. We demonstrate the power of these methods to characterize minor PUFA FAME with subtle differences in double bond position.

**Evaluation of Non-comprehensive Multidimensional Approaches for the Quantification of Fatty Acids Methyl Esters.** Pierluigi Delmonte, US Food and Drug Administration, USA; Sarah Prebihalo, US Food and Drug Administration, USA; Andrea Milani, US Food and Drug Administration, USA

The complexity of fatty acid methyl esters (FAME) analysis depends on which matrix is examined and what information is targeted. While the quantification of the main components of common fats and oils may be achieved by gas-liquid chromatography (GLC), including the use of packed columns, the accurate quantification of analytes including trans fatty acids and conjugated linoleic acid may become remarkably challenging. Gas chromatography using capillary columns of different polarities, and liquid chromatography using different separation chemistries, may be combined in two-dimensional separation schemes to achieve the most challenging quantifications. In this study, the separation principles and selectivity for FAME of each technique is carefully evaluated and the results are used to define which separation methods may be combined to measure specific groups of FAME. Silver ion liquid chromatography affords selectivity towards the number and geometry of FAME double bonds, but not their chain-length, providing high orthogonality when combined with the highly polar GLC capillary columns. Alternatively, using two-dimensional gas chromatography in heart-cut mode, a poly-ethylene glycol and a poly(biscyanopropyl siloxane) GLC capillary column may be combined in a single column set, instead of merging the results from their independent analyses. Lastly, two identical capillary columns maintained at different temperatures may be used by exploiting the dependence of highly polar capillary column selectivity on the elution temperature. The proposed methods, based on either liquid chromatography combined with gas chromatography or gas chromatography using two capillary columns of different polarity, are used to analyze common fats and oils.

**Two- and Three-dimensional Liquid Chromatography with Quadruple (x4) Parallel Mass Spectrometry for Triacylglycerol Analysis.** William Byrdwell, USDA ARS BHNRC MAFCL, USA

The compositions of triacylglycerols (TAGs) in natural seed oils and biological samples such as cow's milk can be exceedingly complex. One-dimensional HPLC with mass spectrometry (MS) detection is often not sufficient to provide complete resolution of isomers or TAGs having the same equivalent carbon number (ECN), and regioisomers are usually not separated. Recent developments in commercially available two-dimensional liquid chromatography (2D-LC) systems have opened new opportunities for resolution of previously inseparable molecular species. We have used orthogonal separation techniques, specifically non-aqueous reversed-phase (NARP) HPLC, which separates TAGs by ECN, coupled with silver-ion UHPLC, which separates TAGs by degree of unsaturation and its locations in the molecules, allowing physical separation of regioisomers. In such cases, two mass spectrometers with complementary ionization techniques monitored the first dimension and two more mass spectrometers monitored the second dimension for LC1MS2 x LC1MS2 = LC2MS4. For the most complex samples, which contain both short-chain and long-chain TAGs, we employ three dimensions of separation, in which the first dimension partially resolves molecular species, and two parallel second dimensions allow greater separation, for LC1MS2 x (LC1MS1 + LC1MS1) = LC3MS4. The first second dimension separates short-chain and long-chain TAGs very well, and the second (parallel) second dimension employs multi-cycle, or "controlled wraparound", chromatography to further resolve ECN isomers. These

experiments not only show the possibilities and benefits of three-dimensional chromatography, but also show the great advantage of multi-cycle chromatography to improve resolution and optimally fill the separation space available in a second dimension.

**Unusual Long Chain Fatty Acids in Sorghum Wax.** Robert Moreau, USDA ARS ERRC, retired, USA; Alberto Nunez, Eastern Regional Research Center, ARS, USDA, USA; Andrew Harron, Eastern Regional Research Center, ARS, USDA, USA; Megan Hums, Eastern Regional Research Center, ARS, USDA, USA; Michael Powell, Eastern Regional Research Center, ARS, USDA, USA

The surfaces of many plant parts are often coated with various types of epicuticular waxes. These waxes provide protection from physical injury, insects, microbes, and desiccation. Both the concentration and chemical composition of epicuticular waxes vary greatly depending on species and on the anatomical plant part (leaf, flower, stem, seed, etc.). The most common chemical components in epicuticular waxes include wax esters (compounds comprised of a fatty acid esterified to a fatty alcohol) and alkanes, but can also include many other types of lipids. The epicuticular wax covering sorghum kernels contains a complex mixture of many types of lipids but the major components are long chain (C28 and C30) fatty acids and fatty aldehydes. The structure and function of these unusual fatty acids will be discussed and compared to the waxes in other grains.

## General Analytical Methods I

*Chairs Lisa Clement, Cargill Inc., USA; Pierluigi Delmonte, US Food and Drug Administration, USA*

**A Fully Integrated Lc-gc×gc-tofms/fid Platform for Mosh & Moah Determination in Food.** Giorgia Purcaro, University of Liège, Belgium; Grégory Bauwens, MA, University of Liege, Belgium; Maurine Collard, University of Liege, Belgium; Sebastiano Panto, LECO European Application and Technology Center (EATC), Germany

The analysis of mineral oil hydrocarbons (MOH) in food is a challenging task, mainly due to the high complexity of the matrices and the high affinity of mineral oil with the lipid fraction and its components. LC-GC-FID is the method of election for the quantitative determination of MOH in the two main fractions, namely saturated (MOSH) and aromatic (MOAH) hydrocarbons. However, this technique provides a chromatogram characterized by an unresolved complex mixture (UCM) from which no confirmatory information of the identity of the targeted compounds can be obtained. On the other hand, GC-MS alone fails to work as a confirmatory method due to the very low specificity of the MS fragments. In this scenario, GC×GC-ToFMS/FID is the most promising solution to characterize the MOSH and MOAH fraction in detail and to answer to the request of the EFSA and the European Union to characterize more in detail the 3–7 ring MOAH fraction.

Within this context, an additional step forward is proposed in order to merge the routine and confirmatory method in a single analysis. Indeed, the use of a fully integrated and automated platform, namely LC-GC×GC-ToFMS/FID was explored and optimized. The qualitative information obtained in the MS trace was translated into the FID trace for quantitative purposes. A novel software algorithm was also developed in order to improve the reliability of GC×GC quantification in MOSH and MOAH where a different quantification logic is needed.

**Characterization and Quantification of a Model Terpene in Oil Using Raman Spectroscopy and Multivariate Approaches.** Jonathan Andrade, Ryerson University, Canada; Jeffery Powell, Ryerson University, Canada; Dérick Rousseau, Ryerson University, Toronto, Canada

This study evaluated the potential application of Raman Spectroscopy to quantify limonene, a model terpene, dissolved in canola oil at concentrations of 0.1 to 1 wt %. Principal Component Analysis (PCA) was used to characterize the initial Raman spectra followed by Partial Least Squares (PLS) regression to model and predict the concentration of limonene in the samples. Analysis of the spectra and their second derivatives showed that limonene's characteristic ring deformation vibration at 750–770 cm-1 resulted in a concentration-dependent photon count whereas the characteristic bands of canola oil were expectedly not affected. Via PCA, three spectral regions weighted significantly and allowed for differentiation of the oil mixtures. PLS yielded low prediction errors, high precision and coefficients of determination with global fit above 0.99. Overall, the use of Raman spectroscopy and multivariate analysis was used to predict the presence and concentration of a model terpene in oil.

**Chemical Fingerprinting of MOH of Lubricants Used in the Coconut Oil Supply Cain by Gpc-hplc-fluorescence/elsd.** Carlos Martin Alberca, Cargill, Netherlands; Bram Dijke, Cargill, USA; Falk Bruse, Cargill, USA; Marian Steverink, Cargill, USA

Edible oils and fats, like other foods, might be contaminated with mineral oil (MO) products along supply chains and production processes as a consequence of bad practices during manufacturing or transport, accidental leaks, contact with certain packaging, or environmental exposure. NGOs and consumer organizations are seriously concerned since there is a huge lack of knowledge about the health effects of exposure to some MO compounds. Therefore, regulatory agencies and industry have reacted, studying and taking control of this issue via several

strategies. One of them is to acquire a better understanding of the potential contamination sources along supply chains and to get more knowledge about the mineral oil hydrocarbons (MOH) linked to these sources. If the source is pinpointed, then it might be possible to prevent this contamination from happening again.

This communication presents a new screening method by GPC-HPLC-Fluorescence/ELSD developed for the quick chemical characterization of MOH in lubricants used in Cargill's coconut oil supply chain. This study aims to obtain the chemical profile of these MO products to be able to detect them in the event they appear in coconut oil products. For that, first, a sample-prep and a separation approach by GPC-HPLC were tested and optimized for their use with edible oil and lubricants. Next, up to 30 different technical and food-grade lubricants related to the coconut oil supply chain and homemade contaminant-free crude coconut oil were analyzed, aiming to obtain their chemical fingerprints. The obtained chemical profiles showed several characteristic compounds that allowed to determine the specific type of lubricant spiked in a coconut oil sample. This result confirms that the proposed method is a suitable screening tool for detecting this type of MO contamination in coconut oil. This approach can be used to quickly determine the source of lubricant contamination along the supply chain.

**Novel Easy Ultraspeed Determination of More Than 700 Pesticides and 40 Plasticizers in Fats and Oils by Pica Method.** Anna Romanotto, PiCA GmbH Berlin, Germany; Jeanette Langner, PiCA GmbH Berlin, Germany; Martin Sander, PiCA GmbH Berlin, Germany

Conventional method of determining pesticides in fats and oils are based on gel permeation chromatography (GPC) in order to separate both substance classes. Developed in the 1980s, the laborious and inconvenient GPC procedure is still in use today in many laboratories especially for the determination of residues of nonpolar pesticides. The newer extended QuEChERS method, so called QuOil is faster, but not optimally suited for nonpolar pesticides because of their low recovery rate. The Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid) also allows very good cleanup of fatty samples without time consuming GPC.

We developed a completely new and very simple method without GPC and without any expensive SPE materials. The method is based on a combination of several common solvents which have to be applied in both the right combination and order.

- solvent A dissolves/dilutes rapidly oil/fat sample. No separate extraction step is required
- solvent B pre-extracts pesticides and plasticizers from the oil/fat/solvent A mixture
- solvent C separates fat/oil phase from the pesticide/plasticizers extract which can immediately be measured with GC-MSMS, GC-MS and LC-MSMS equipment. The sample preparation is done in 10 minutes.

Besides the method development we also optimized our measurement methods UHPLC-MSMS and GC-MSMS, which contains 714 parameters for pesticides and the GC-MS method for the determination of 40 plasticizers with the required LOQs.

Our novel procedure is fully validated (SANTE/12682/2019) for an oil mixed matrix made up of different crude oils. The quality and correctness of the method have been 100% confirmed in annual FAPAS ring tests since it was developed in 2015. The method has also been successfully tested with usually problematically oils such as fish oil, argan oil and animal fats such as milk fat. This procedure has been accredited at PICA since 2017 (by DAkkS) and is in use.

**Separation of Fatty Acid Isomers in Human Plasma by Silver Ion High-performance Liquid Chromatography in Conjunction with Gas Chromatography with Negative Chemical Ionization Mass Spectrometry.** Na Wei, CDC, USA; Hayoung Kim, CDC, USA; Jessica Holmes, CDC, USA; Heather Kuiper, CDC, USA; Hubert Vesper, CDC, USA

Trans-fatty acid (TFA) consumption is associated with increased risk of cardiovascular disease, the leading cause of death worldwide. Reliable and reproducible measurements of TFA in blood are necessary to monitor levels of TFA in people, compare blood TFA levels among different geographical regions, and to better assist regions with policy evaluation to eliminate industrially produced TFA and therefore reduce cardiovascular disease risk.

A large number of different TFA have been reported in food and are anticipated to occur in blood. However, limited information is available about the range of different TFA in blood. Furthermore, oils used in different regions in the world may produce different TFA isomers. This may result in individuals from different geographical regions being exposed to different TFA isomers. To obtain better, more comprehensive information on TFA isomers occurring in blood, a new analytical approach is needed that can measure a broad range of TFA isomers in blood.

We have developed a method using silver ion high-performance liquid chromatography (Ag-HPLC) in conjunction with gas chromatography-negative chemical ionization-mass spectrometry (GC-NCI-MS) adopted from the CDC isotope-dilution GC-NCI-MS for the quantitation of fatty acids in human plasma. This approach allows us to fully separate TFA from their cis-isomers. With this method, we were able to detect over 140 fatty acid peaks in human plasma. Fifteen of them have been fully confirmed and verified with their positional and geometric configuration of double bond. To the best our knowledge, three TFAs (C16:1n-5t, C17:1n-7t, C22:1n-9t) have not previously been reported in human plasma. Knowledge about these TFA in human blood may allow for better understanding

of health risks associated with TFA exposure and provide information about potential regional differences in TFA exposure.

**What Can Go Wrong When You Measure Colour.** Peter Clarke, The Tintometer Limited (Lovibond), United Kingdom; Matthew Russell, The Tintometer Limited (Lovibond), United Kingdom

Defining the colour of a product accurately and consistently is of vital importance in the edible oil and fat industry. The colour of transparent samples is most commonly expressed in Lovibond® RYBN colour, or in certain markets, AOCS-Tintometer® colour.

Clear and correct description of colour standards and tolerances is critical when:

- Specifying materials when sourcing.
- Communicating colour within the wider supply chain.
- Inspecting incoming materials.
- Conducting continual production quality control.
- Inspecting final/outgoing products.
- Guaranteeing compliance with national and international standards.

Reliable and repeatable colour test results are key to ensuring final product quality and to minimizing production costs. Speed of analysis can also be vital for efficient process control. Simplicity of operation helps to reduce errors and increase productivity.

Although clearly defined in AOCS Standards such as Cc 13j-97 and AOCS Cc 13e-92, common misunderstandings and well-intended 'short-cuts' have become common practice in many edible oil and fat quality control laboratories.

This presentation will summarize these issues and explain how to adapt or more preferably avoid them. We will share new analytical data supporting the advice we provide.

## General Analytical Methods II

*Chairs Lisa Clement, Cargill Inc., USA; Pierluigi Delmonte, US Food and Drug Administration, USA*

**Characterization of Italian Evoos Blends, Using 1H NMR Spectroscopy Databases of Monocultivar Reference Oils and Statistical Models.** Francesco Paolo Fanizzi, University of Salento, Italy; Chiara Roberta Girelli, University of Salento, Italy; Francesca Caló, University of Salento, Italy; Federica Angilé, University of Salento, Italy; Lucia Mazzi, Certified Origins, Italy; Daniele Barbini, Certified Origins, Italy

During the last few years, the global demand for extra virgin olive oil (EVOO) is increased. Olive oil represents an important percentage of world fat consumption determining a significant development of its market. In this context, the problems related to counterfeiting and product fraud is becoming extremely relevant. Thus, the quality and authenticity control of EVOOs is actually a hot topic issue. In this work we focused on the use of 1H Nuclear Magnetic Resonance (NMR) technique associated with multivariate statistical analysis (MVA) to characterize Italian EVOOs Coratina based commercial blends. A specific database including monocultivar EVOOs reference samples, was used to characterize Italian EVOOs blends over four consecutive harvesting years. Moreover, the contribute of the minor components (phenolic compounds) on the qualitative characteristics of blended EVOOs was also evaluated. The correlation analysis of classification scores obtained using two pair wise orthogonal partial least square-discriminant analysis (OPLS-DA) models (built with major and combined major–minor components NMR data) revealed that both could be profitably used to generally classify the studied Coratina based blends. This classification may also constitute an indirect method to rank commercial Coratina based blend samples according to their expected bitterness and/or pungency characteristics. Therefore, tailor-made databases based on 1H NMR data of monocultivar oils from specific geographical origins could be profitably used to build a gate around high-quality blend EVOOs and define their characteristics with respect to a specific monocultivar reference oils dataset.

**Determination of Adulteration of Sunflower Oil with Safflower Oil Using Some Sterol Components and Fatty Acid Compositions.** Fatma Nevin Başaran, Besler Gıda ve Kimya San ve Tic. A.Ş., Turkey; Aziz Tekin, Ankara Unıversity, Turkey; Murat Tasan, Namık Kemal University, Faculty of Agriculture, Turkey; Yusuf Özgür Anuk, Besler Gıda ve Kimya San. ve Tic. A.Ş., Turkey; Ferda Altuner, Besler Gıda ve Kimya San. ve Tic. A.Ş., Turkey

Vegetable oils are very important energy source for human body and their authenticity has become a major issue for the producers, consumers, and policy makers. Adulteration, a type of fraud, is performed by addition of cheap oils to the expensive ones. Although many methods have been available to detect the adulterations in vegetable oils, it has still been an important problem for all stakeholders. While olive oil is the most adulterated vegetable oil due to its high price, the others can also be subjected to adulteration depending on their market price.

Sunflower oil is one of the most consumed seed oils in the World, but it can also be adulterated by some other cheaper oils such as safflower oil. Since both oils have similar fatty acid compositions, detection of safflower oil in sunflower oil is a big challenge.

In this study, some blends in certain proportions of crude safflower oil: crude sunflower oil (2:98, 4:96, 6:94, 8:92, 10:90, 14:86, 18:72, 22:78, 26:74) were prepared. The changes in fatty acid and sterol compositions in these blends were determined. Fatty Acid Composition (FAC) were analysed using gas chromatography (GC) and according to AOCS Ce 1-62 Method

(AOCS 2009), and distribution of sterol components were determined using gas chromatography (GC) by COI/ T.20/Doc. No. 10/ Rev. 1 (2001). All findings were evaluated by Principal Component Analysis (PCA).
The results showed that some fatty acids and sterols could be good indicators to detect the adulteration of sunflower oil with safflower oil. PCA Analyses indicated that safflower oil can be detected in sunflower oil as low as 4%.

**Evaluation of Vibrational Spectroscopy Techniques (NIR, MIR, Raman) Combined with Chemometrics in Detecting Adulteration of Essential Oils.** Ahmed Menevseoglu, Gumushane University, Turkey; Cansu Gumus, Ankara University, Turkey
Oil adulteration is a growing concern worldwide. Essential oils such as sesame, poppyseed, linseed oil etc. are a target for economically motivated adulteration due to their high commodity value. Some of essential oils contain high concentrations of healthy compounds such as omega-3 fatty acids which plays an important role in reducing cardiovascular diseases. Therefore, the high demand for essential oils is due to their health-related benefits. Fixed oils are the adulterants in essential oils due to the similar fatty acid profiles. A rapid method to detect essential oil adulteration is in need as they are relatively more expensive than fixed oils. Various chemical methods have been used to authenticate essential oils; however, traditional methods such gas chromatography, high performance liquid chromatography, and mass-spectroscopy are requiring high-cost instrumentation and maintenance, and well-trained personnel to operate the instrumentation, and also being time consuming makes them less appealing as a technique to authenticate the oils. Vibrational spectroscopy techniques are easy to operate, non-invasive, and rapid for real-time assessments as well as cheaper alternatives to traditional methods. With the development of technology, handheld and portable FT-NIR, FT-MIR, and Raman spectrometers are available in the market. Portable vibrational spectrometers can be used for the authentication of essential oils. These units provide sensitive, high throughput, and rapid information of the essential oils and their possible adulteration. Vibrational spectroscopy techniques show great potential for real time surveillance to detect fixed oil adulteration in essential oils.

**Identifying ‘‘green’’ Solvents for the Electrochemical Quantitation of Antioxidants.** Jaycie Montney, University of Michigan–Flint, USA; Matthew Fhaner, University of Michigan–Flint, USA
Recent work within our research group has focused on utilizing electrochemical methods, such as square wave voltammetry, for the quantitation of antioxidants. Of specific interest, is using electrochemistry as a supplemental technique for assessing the amount of unoxidized antioxidant species in consumer products. Electrochemical methods lend themselves to fast analysis times (< 2–3 minutes per sample), low detection limits (1–50 μM for investigated antioxidants), and utilizes rugged instrumentation that can be portable. However, the solvent systems historically used in electrochemical studies have relied on hazardous solvents such as hexanes and benzene. Before the applications of electrochemical methods can fully be realized, ‘‘greener’’ solvent systems must be identified. Therefore, solvents that were less hazardous, in ready supply, and could reduce potential waste were investigated to determine how each solvent system combination affected the figures of merit for three antioxidant systems. The specific solvent systems investigated were ethanol, acetonitrile, ethyl ether, and isopropanol combined in ratios of 1:3, 1:1, 3:1, and pure solvent. Limits of detection, lower limits of quantitation, and calibration linearity will be presented for a synthetic antioxidant (butylated hydroxytoluene) and two natural antioxidants (sesamol and rosemary extract).

**Mitigation of Avocado Oil Adulteration—the Food Chemicals Codex Identity Standard.** Gina Clapper, Food Chemicals Codex, US Pharmacopeia USA; Tongtong Xu, USP, USA
Avocado is obtained from the fruit of avocado plant (Persea americana Mill.) by means of extraction using mechanical pressing, solvents, or enzymes. Recently avocado oil has generated increasing interest and popularity among consumers due to the perceived nutritional benefits of high oleic acid and antioxidants in the oil. The global market size for avocado was 499 million US dollars in 2019 and is expected to reach 673.6 million US dollars by the end of 2026. Because of its popularity and higher unit price compared to other conventional food oils, avocado oil is vulnerable to economically motivated adulteration. A recent study found that most avocado oil commercial products in the US market were of poor quality and oxidized before reaching the listed expiration date. Adulteration with soybean oil was detected at levels near 100% in some refined avocado oil products. Lack of an international quality standard for avocado oil leaves consumers with insufficient protection from products adulterated with low-cost/quality oils. To meet the growing need for an independent standard for avocado oil, the Food Chemicals Codex (FCC) is developing an Identity Standard which will describe identification and quality parameters for avocado oils. The analytical methods and specifications in this standard will be developed using relevant industry experimental data, scientific literature, consumer product data and industry information from various producing countries. This new FCC Identity Standard, expected to be proposed in 2021, will better help industry and regulators combat adulteration, protect consumers and support healthy growth of the avocado oil industry.

## Rapid and High-Throughput Screening Methods

*Chairs David Barr, Bruker BioSpin Corp., USA; Walter Vetter, University of Hohenheim, Germany*

**Applications of Nuclear Magnetic Resonance Spectroscopy for Food Authenticity Control.** Eva Gottstein, CVUA Karlsruhe, Germany; Dirk Lachenmeier, CVUA Karlsruhe, Germany; Thomas Kuballa, CVUA Karlsruhe, Germany

Food authenticity is a complex issue with multiple aspects, for example the geographical origin of products, more precisely geographical indications such as Protected Designation of Origin (POD) and Protected Geographical Indication (PGI). Products with such specifications have unique characteristics and a high quality and, therefore, they are susceptible to food fraud. However, there are many possibilities to fraud food including unapproved treatments, falsification, brand fraud, denomination of origin, or claiming conventional products to be organic. Replacing one or more components with cheaper ones that have equivalent properties is the most common way of food fraud. Due to the steady rising of food fraud, reliable analytical tools are necessary to reveal fraudulent processing and adulteration.

Food products are known to comprise complex matrices with a huge number of different compounds in diverse concentrations. Nuclear magnetic resonance (NMR) spectroscopy allows with a single spectrum an insight into these matrices similar to a ‘‘fingerprint’’ which is specific to the respective product. To handle and evaluate the immense amount of data generated by NMR spectroscopy and to filter out the most relevant information of a spectrum, multivariate data analysis such as principal component analysis (PCA) combined with linear discriminant analysis (LDA) is required.

NMR spectroscopy in combination with multivariate data analysis has been used to analyze various food matrices in terms of authenticity. It was shown, that application of NMR is suitable to differentiate the botanical origin of honey and to identify the geographical origin of juice. Beside geographical aspects, adulteration of coffee was investigated with NMR techniques and chemometrics. Additionally, this method was used to differentiate organically and conventionally produced chicken eggs as well as organically and conventionally produced milk in a reliable and powerful way.

**Evaluation of Micro-ESR for Monitoring Lipid Oxidation in Oils and Powdery Matrix.** Francesca Giuffrida, Nestle Research Ctr, Switzerland; Pierre-Alain Golay, Nestle Research, Switzerland; Emmanuelle, Bertschy, Societes des produits Nestlé, Switzerland

Lipid oxidation impacts food quality through flavor and taste deterioration often at the origin of consumer complaints and causing also a decrease in nutritive value.

Several technologies have been developed to monitor lipid oxidation. However, these technologies can be time consuming, require the use of chemicals and mostly were developed to measure lipid oxidation in oils and fats.

In this study, micro-ESR was evaluated as rapid, safe and user-friendly technology for the assessment of lipid oxidation in ingredient and finished product.

Micro-ESR allowed rapid monitoring of oxidation in oil used as ingredient and finished product (i.e., Infant Formula) with minimal sample pre-treatment, showing repeatability and intermediate reproducibility values lower than 15%.

**In-the-field Determination of Free Acidity in Olive Oil Using a Portable Battery-operated Sensor System.** Marco Grossi, University of Bologna, Italy; Enrico Valli, Alma Mater Studiorum - Università di Bologna, Italy; Alessandra Bendini, Alma Mater Studiorum - Università di Bologna, Italy; Tullia Gallina Toschi, Alma Mater Studiorum - Università di Bologna, Italy; Bruno Riccò, University of Bologna, Italy

Olive oil quality grade is assessed by legal conformity check of chemical and sensory parameters and free acidity is the first of them. The official method to determine free acidity is by means of a titration carried out in a proper laboratory by trained personnel.

An alternative technique that can provide fast and accurate determination of oil free acidity is the electrical conductance measurement of an emulsion between an hydro-alcoholic solution and the sample under test. This method has the advantage to be implementable in automatic form and to use nontoxic reagents that can be easily disposed of.

A portable battery-operated sensor system has been designed and built making possible the free acidity measurement in about 30 seconds. The proposed system has been in-house validated using 30 olive oil samples with a satisfactory performance in terms of LOD, LOQ, precision and accuracy. This tool can be used on-site in production environment (e.g., oil mills or packaging centers) by anyone. The system (with dimensions 11 x 15 x 5 cm) can be powered by 3 AAA alkaline batteries or by a PC USB port. It is made of a PCB electronic board that integrates a microcontroller (STM32L152RCT6A) and all the electronics to perform the measurements. The sample is hosted in a modified 50 mL vial that features two cap-shaped stainless steel electrodes for the electrical conductance measurement.

The proposed instrument is potentially of great interest for small production centers that cannot afford an internal laboratory for quality analysis and send the samples to an external laboratory with high costs and time needed.

This work was developed in the context of the project OLEUM ‘‘Advanced solutions for assuring authenticity and quality of olive oil at global scale funded by the European Commission within the Horizon 2020 Programme (GA no. 635690).

**Microesr to Check the Peroxide Value in Virgin Olive Oils.** Matilde Tura, Alma Mater Studiorum-Università di Bologna, Italy; Mara Mandrioli, Alma Mater Studiorum-Università di Bologna, Italy; Enrico Valli, Alma Mater Studiorum - Università di Bologna, Italy; David Barr, Bruker BioSpin Corp., USA; Manfred Spraul, Bruker BioSpin GmbH, Germany; Agnes Haber, Bruker BioSpin GmbH, Germany; Alessandra Bendini, Alma Mater Studiorum - Università di Bologna, Italy; Tullia Gallina Toschi, Alma Mater Studiorum - Università di Bologna, Italy

Lipid autoxidation proceeds by the formation of free radicals and, as a consequence of stressful conditions during processing or storage of edible oils. For the quality control laboratories, it is necessary to easily and quickly check oxidative state markers, such as peroxides (PVs), to analyze many samples in a short time. The present study aimed to test electron spin resonance (ESR) spectroscopy as a screening, automated and solvent-efficient tool to assess the oxidative state of virgin olive oils (VOOs), by finding correlation models between PVs, determined by the EU official method, and ESR data. Two linear regression models were built by plotting the PVs of the samples and the concentration of free radicals at two different times, by a specific ESR assay conducted on 35 VOOs. The PVs and the ESR concentration of free radicals of 7 VOOs (3 for internal and 4 for the external validation) were determined. To test the accuracy of the two models, results of the ESR assay were interpolated in the two equations, and ESR predicted PVs were compared to those obtained by the official method. The two methods were statistically consistent (Student's test, $p \leq 0.05$). These outcomes support the use of ESR as a sustainable screening tool to control the primary oxidation products in VOOs. The ESR procedure needs a low amount of chemicals, compared to the large quantities required by the EU official method for the PVs determination. To reach a high predictive ability of the ESR procedure for VOOs it will be necessary to analyze a higher number of selected samples and to investigate if and when it is necessary to consider specific correction factors for the prediction. The latter can be needed when linoleic or linolenic acids are high or with exceptionally high or low polyphenol content.

**Rapid Analysis of Ruminant Fat Adulteration by Spectroscopy Combined with Machine Learning.** Pei-Jin Tong, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China (People's Republic); Hong-Chao Zhang, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China (People's Republic); Ting-Ting Wei, Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd, China (People's Republic); Wen-Ming Cao, Wilmar Intl, China (People's Republic)

Beef tallow (BT) is a representative ruminant fat with high melting point and has been widely used in food industry, such as bakery products, shortening, margarine and hotpot seasoning. BT is rich in a variety of vitamins, minerals, cholesterol and high in saturated fatty acids, which has good stability and economic values. Due to the price differences and lacking of detection and supervision methods, 52°C palm stearin (ST) or mutton fat (MF) are prone to be added into BT products to improve melting point and reduce costs. However, to the best of our knowledge, no studies regarding the adulteration of BT samples or products using NIR spectroscopy have been published. This study presents an effective rapid method for qualitative and quantitative analysis of BT adulteration by using near infrared spectroscopy (NIR) combined with machine learning technology. Prue BT can be easily identified from adulterated BT with ST and MF by adapting PLS-DA method with an accuracy of 100% and 98.2%, respectively. PLS method was used for building quantitative models. The quantitative model of analyzing ST content from adulterated BT-ST samples was successfully established with R2 of 0.9989, root mean square error of cross validation (RMSECV) as 0.882% and root mean square error of prediction (RMSEP) as 1.65%. The quantitative model of analyzing MF content from adulterated BT-MF samples was also established with R2 of 0.9998, RMSECV as 0.429% and RMSEP as 0.469%. The research showed that NIR spectroscopy combined with machine learning has the potential to be used as an economic, rapid, non-destructive and quantitative technique for testing authenticity of ruminant fat samples.

**Rapid Non-destructive Analysis of Intact Canola Seeds Using a Handheld Near-infrared Spectrometer.** Veronique Barthet, Canadian Grain Commission, Canada; Michael W.P., Petryk; Canadian Grain Commisssion; Canada Bert Siemens, Canadian Grain Commission, Canada

The NIR models for canola quality were developed with samples from Canadian canola seeds harvested in 2016 and 2017. All calibration models were first tested on a 2017 external validation sample set. The handheld NIR spectrometer used in this study has a limited wavelength range 908.1 nm to 1676.2, however, the validation results showed that it could be used to predict several important parameters that defined canola seed quality. Final testing was done using calibration models with the least number of factors on a second external canola validation sample set (2018 harvest). Some calibration models showed excellent stability and predictive powers with R2val values of 0.94 to 0.99 (i.e., oil, protein, oleic acid and iodine value) and low SEPs for both external validation sample sets. The a-linolenic acid model had an R2val of 0.93 when applied to the 2017 external validation set, the correlation fell slightly

to 0.88 when applied to the 2018 external validation sample set, potentially indicating a slight instability in the model. The prediction model for total glucosinolate was not very good, but still could be used to segregate the samples into low or high glucosinolate samples. Finally, the predictive models for chlorophyll and total saturates were unusable. The chlorophyll model was very unstable, likely due to the instrument's limited wavelength range.

## Trace Contaminants

*Chairs Jessica Beekman, US Food & Drug Admin, USA; Jan Kuhlmann, SGS Germany GmbH, Germany*

**An Update on EU Regulations for Free/bound 3-MCPD and Glycidol in Oils and Infant Formula.** Jessica Beekman, US Food & Drug Admin, USA

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in a variety of edible oils and and food products containing these oils. Formed during the high temperature deodorization step of edible oil refining, these contaminants may be carcinogenic and/or genotoxic making their presence in foodstuffs a potential health concern. In 2018, the European Union established regulations for maximum levels of glycidyl esters (expressed as bound glycidol) in edible oils and infant formula. And more recently, in January 2021, these regulations were expanded to also include maximum levels for free and bound 3-MCPD. This presentation will discuss these important regulations as well as their influence and implications — not only in the EU, but in other parts of the world as well.

**Chloroparaffins: Widespread Process Contaminants of Edible Oils.** Kerstin Krätschmer, EURL POPs, Germany; Walter Vetter, University of Hohenheim, Germany; Alexander Schächtele, European Union Reference Laboratory for Halogenated POPs in Feed and Food (EURL POPs), Germany

Chloroparaffins (CPs) are current-use polyhalogenated chemicals whose production volume already exceeds the one of polychlorinated biphenyls (PCBs) by one order of magnitude. Chloroparaffins are highly complex mixtures of ~10,000 compounds that are synthesized by the chlorination of alkane feedstocks. According to carbon chain length, they are distributed into three classes, i.e., short-chain chloroparaffins (SCCPs, polychlorinated decanes to tridecanes) which were recently classified as persistent organic pollutants (2017) as well as currently nonregulated medium-chain chloroparaffins (MCCPs, polychlorinated tetradecanes to heptadecanes) and long-chain chloroparaffins (LCCPs, polychlorinated octadecanes and longer alkanes). CPs are used both as flame-retardants and lubricants in various industrial processes and products. Due to their lipophilic character and the common use of lubricants in oil production, CP-containing oil presses may release CPs during their use followed by leaching of CPs into (edible) oils.

Cooking oils and refined oils (n=31) showed CP levels between < LOD and 150 μg/kg. Even higher maximum concentrations were determined in cold-pressed olive oil (n=13) with 13–1900 μg/kg. Our findings indicate that CPs are (i) introduced during the pressing of the oil and (ii) partly removed during oil refining. The wide contamination ranges indicated that CP levels of edible oils can be reduced by controlling the technical processes. A recent market basket study in Germany showed that edible oils are currently the food group with highest CP levels. Hence, oils are suspected to contribute significantly to the current CP intake of humans via contaminated food.

**Fatty Acid Esters of Glycidol and 2- and 3-monochloropropanediol (2- and 3-MCPD) in Food: What About Internal Exposure in Humans?** Bernhard Monien, German Federal Institute for Risk Assessment, Germany; Klaus Abraham, German Federal Institute for Risk Assessment, Germany

Fatty acid esters of glycidol, 2-monochloro-1,3-propanediol (2-MCPD) and 3-monochloro-1,2-propanediol (3-MCPD) are processing contaminants generated during deodorization of vegetable oils or during other heating processes. The hydrolysis in the gastrointestinal tract releases glycidol and 2/3-MCPD. Due to the difficulties of external exposure assessment, we developed analytical techniques for the quantification of biomarkers of internal exposure for glycidol and 2/3-MCPD.

The reactive glycidol forms adducts with DNA and proteins, one of which is 2,3-dihydroxypropyl-Val (DHP-Val) at the N-terminus of hemoglobin. A controlled exposure study was conducted with 11 non-smoking participants consuming a daily portion of about 36 g commercially available palm fat (8.7 mg bound glycidol/kg) over 4 weeks. In this period and the following 15 weeks DHP-Val was monitored using an Edman degradation and UHPLC-MS/MS. During the intervention, the mean adduct level increased from 4.0 to 12.2 pmol DHP-Val/g hemoglobin and then decreased to the background level in a linear fashion, demonstrating the suitability of DHP-Val as biomarker at least in case of relatively high exposure.

In contrast to glycidol, 2/3-MCPD are less reactive and are partly excreted unchanged in the urine within 24 h after exposure. In order to study whether urinary 2/3-MCPD are possible biomarkers of short-term exposure, we determined the complete excretion of 2/3-MCPD on four consecutive days before and after consumption of 12 g 2/3-MCPD ester-rich hazelnut oil (bound 3-MCPD 54.5 mg/kg, bound 2-MCPD 24.2 mg/kg) in 12 non-smoking participants. The mean

excretion amounts were 14.3% (2-MCPD) and 3.7% (3-MCPD) of the study doses. Using these factors for reverse dosimetry, the average daily 'background' exposure was estimated to be 0.12 and 0.32 μg/kg body weight for bound 2-MCPD and 3-MCPD, respectively. The agreement with exposure estimates from the EFSA indicated that urinary 2/3-MCPD are suitable biomarkers for the external exposure to the respective fatty acid esters.

**Mitigation of 3-MCPDE & GE in Refined Palm Oil.** Yen Li Yung, IOI Edible Oils Sdn Bhd, Malaysia; Shyam Lakshmanan, IOI Edible Oils Sdn Bhd, Malaysia

3-MCPDE and GE are food-borne contaminants that are formed from monoglycerides (MAG), diglycerides (DAG) and triglycerides (TAG) that are natural components of edible oils. During thermal processing of edible oils containing chlorides, 3-MCPDE may be formed from DAG or TAG. Chloride reduction in crude palm oil (CPO) of 74–80% was achieved with hot water washing. Chloride levels below 1.5 mg kg-1 were achieved in refined palm oil upon refining of washed CPO containing less than 2 mg kg-1 chloride. Such chloride levels resulted in 3-MCPDE levels of 1.3 mg kg-1 in refined palm oil. CPO washing was observed not only to reduce chloride content, but also aided in reducing phosphorous content by 20%. Trace elements such as iron, calcium, magnesium were also reduced in the washing operation by 18%, 27% and 42% respectively. GE is formed from DAG or MAG when the oil is heated, and the rate of formation increases when the temperatures exceeds 230°C. Utilization of lower deodorization temperatures in lab scale experiments can produce refined palm oil with GE levels close to 1 mg kg-1 using premium quality CPO. Reduction of GE by 23–54% has been achieved through post refining treatments using various techniques. Conducting post refining treatment at 110°C, GE levels below 1 mg kg-1 were achieved using adsorbent X. The research shows that chloride reduction can mitigate 3-MCPDE formation while post refining treatment can reduce GE in refined palm oil.

**Simultaneous Analysis of Malondialdehyde, 4-hydroxy-2-hexenal, and 4-hydroxy-2-nonenal in Vegetable Oil by Reversed-phase High-performance Liquid Chromatography.** Guoqin Liu, South China University of Technology, China (People's Republic); Lukai Ma, Zhong Kai University of Agriculture and Engineering, China (People's Republic)

Malondialdehyde (MDA) and α,β-unsaturated aldehyde such as 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE) are formed among other saturated or unsaturated aldehydes. MDA is known to be formed from both ω-3 and ω-6 PUFAs and has been claimed to be the most important end-product of lipid autoxidation. While HHE and HNE are related to the oxidation of ω-3 and ω-6 PUFA, respectively. MDA, HHE, and HNE have been identified as the toxic substances. They have been considered to cause several diseases such as adult respiratory distress syndrome, atherogenesis, diabetes, and even cancer. Then simultaneous determination of them holds a great need in both the oil chemistry field and food field. In the present study, a simple and efficient analytical method was successfully developed for the simultaneous separation and detection of MDA, HHE, and HNE in vegetable oils by reversed phase-high-performance liquid chromatography (RP-HPLC) coupled with photodiode array detector (PAD) at dual-channel detection mode. The effect of various experimental factors on the extraction performance, such as coextraction solvent system, butylated hydroxytoluene addition, and trichloroacetic acid addition were systematically investigated. Results showed that the linear ranges were 0.02–10.00 μg/mL for MDA, 0.02–4.00 μg/mL for HHE, and 0.03–4.00 μg/mL for HNE with the satisfactory correlation coefficient of >0.999 for all detected aldehydes. The limit of detection (LOD) and limit of quantification (LOQ) of MDA, HHE, and HNE were ∼0.021and 0.020 μg/mL, ∼0.009 and 0.020 μg/mL, and ∼0.014 and 0.030 μg/mL, respectively. Their recoveries were 99.64–102.18% 102.34–104.61% and 98.87–103.04% for rapeseed oil and 96.38–98.05% 96.19–101.34% and 96.86–99.04% for French fries, separately. Under the selected conditions, the developed methods were successfully applied to the simultaneous determination of MDA, HHE, and HNE in different tested vegetable oils. The results indicated that this method could be employed for the quality assessment of vegetable oils.

**Towards Harmonized Methods for Determining MOSH/MOAH in Challenging Food Matrices.** Stefanka Bratinova, JRC, European Commission, Belgium; Jan Kuhlmann, SGS Germany GmbH, Germany

The analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in food, especially in food with a high fat content, is very demanding in terms of analytical methodology and interpretation. As MOSH and MOAH are operationally defined parameters, i.e., the reported result depends on the analytical procedure from sample preparation to data evaluation, the method for their determination requires harmonisation regarding definitions, general analytical procedure, performance characteristics and data reporting. In this frame, a guidance document has been published by the European Union Reference Laboratory for Food Contact Materials (EURL FCM) in 2019 to support a European Commission (EC) Recommendation for monitoring the presence of mineral oil hydrocarbons in food in order to collect sufficient occurrence data for risk assessment.

Meanwhile in November 2019, a dispute emerged related to published data on MOAH in infant formula.

The comparability and thus reliability of the results from different analytical procedures applied by laboratories was in question. It should be noted that IF is one of the most challenging matrices in terms of composition and MOAH interpretation.

The EURL FCM identified the need for a fully harmonised analytical method to be characterised with respect to its performance and further standardised. This was also recognised by all stakeholders Therefore, the EURL FCM has started the harmonisation process on request of the EC.

Current achievements from this process will be presented resulting from:

- A roundtable discussion with all stakeholders;
- An exploratory review and trial of the variety of experimental procedures applied;
- Cooperation with stakeholders for the preparation of suitable test materials for collaborative trials;
- Drafting of a harmonised standard operating procedure (SOP) to be validated;
- Organisation of a pre-trial for the evaluation of the draft SOP;
- Preparation for an upcoming full collaborative trial.

## Contemporary Analysis of Lipid Oxidation Products: Detecting and Quantitating more Products at Lower Levels

*Chairs Marc Pignitter, University of Vienna, Austria; Fernanda Furlan Goncalves Dias, University of California, Davis, USA*

**Charged Aerosol Detectors Facilitate Detection and Quantitation of Lipids and Lipid Oxidation Products Without Chromophores.** Morgan Kandrac, Rutgers University, USA; Karen Schaich, Rutgers University, USA

Analyses of lipid oxidation products by high pressure liquid chromatography are hampered by lack of chromophores for detection, lack of pure compounds and extinction coefficients for preparation of standard curves, and long retention times of triacylglycerols. This paper reports how charged aerosol detectors combined with normal phase HPLC can provide advantages in detecting and quantitating all non-volatile oxidation products (provided they can be separated in normal phase mode), providing low detection limits, a wider linear dynamic range than evaporative light scattering and optical detectors, good reproducibility, and distinction between monomer oxidation products, small dimers, and triacylglycerols with and without core oxidation products in a single analysis.

**Early Assessment of Lipid Oxidation in Emulsified Foods.** Marie Hennebelle, Wageningen University, Netherlands; Donny Merkx, Unilever, Wageningen University, Netherlands; John Van Duynhoven, Unilever, Netherlands

Lipid oxidation is a major challenge for food industries, as it leads to the generation of off-flavors and contributes to the reduced shelf-life of lipid-containing products. Shelf-life studies can be time-consuming, even under accelerated shelf-life conditions and it is therefore relevant to develop new methods to predict quickly and accurately the shelf-life of food products. Here, we propose two approaches based on early assessment of either trapped radicals with Electron Spin Resonance (ESR) or hydroperoxides by Nuclear Magnetic Resonance (1H-NMR). N-tert-butyl-α-phenylnitrone (PBN) spin-trapping of lipid radicals in combination with ESR allows the ranking of oxidative stability of emulsions within hours under accelerated shelf-life conditions (50°C). However, our data showed that in bulk oils and emulsified foods, PBN-peroxyl radical adducts are quickly degraded into 2-methyl-2nitrosopropane (MNP), benzaldehyde and alkoxyl radicals. The trapping of peroxyl-radicals and the subsequent generation of alkoxyl-radicals impacts downstream reaction pathways which precludes mechanistic studies and quantitative prediction of downstream appearance of off-flavors. In the 1H-NMR approach, we used a sigmoidal Foubert-adapted model to describe the initial phase of hydroperoxide generation under accelerated shelf-life conditions (50°C). In emulsified foods, the accumulation of hydroperoxides at the interface up to a critical concentration leads to a second acceleration of the lipid oxidation process. Using the point at which the hydroperoxide reaches this critical concentration as a predictor for the onset of aldehyde generation allowed us to predict accurately the aldehyde onset within a week of shelf-life. Moreover, the model parameters could be linked to chemical characteristics of the anti-oxidants present in the product. Altogether, both approaches showed promising results for the early assessment of lipid oxidation in emulsified foods. The 1H-NMR approach presents the advantage to provide a quantitative prediction of aldehyde onset and mechanistical discrimination of antioxidant routes, while the ESR approach only allows a qualitative oxidative ranking.

**Oxylipins Detected for the First Time in the Oxidation Process of Edible Oils Rich in Omega-6 Polyunsaturated Groups by 1H NMR.** Jon Alberdi-Cedeño, University of Vienna, Vienna, Austria/ University of the Basque Country (UPV/EHU), Basque Country, Spain; María Luisa, Ibargoitia, University of the Basque Country (UPV/EHU), Spain

Introduction: Lipid oxidation is one of the main degradative processes occurring in oils and fats. Throughout the lipid oxidation process, not only the reduction of nutritional and sensorial quality take place but also new compounds are generated, some of them with well-known toxic effect for human health. However, in spite of this, certain mechanism through which oxidation

evolves and the identity and concentration of many of the compounds formed, are still unknown nowadays.
Objective: In this context, the aim of this work was to analyze in a global, broad and in-depth way, by means of 1H NMR spectroscopy, the evolution of corn oil oxidation when it is submitted to mild oxidative conditions. Special attention was paid to the identification and formation rate of newly formed oxidation compounds.
Methods: The study was carried out with refined corn oil. Corn oil samples were submitted to mild oxidative conditions. Their study was carried out using 1H NMR spectroscopy, which does not require chemical modification of the sample.
Results: A large number of oxylipins, which contain very varied functional groups such as, hydroperoxy, hydroperoxy-epoxy, hydroxy-epoxy, keto-epoxy, hydroxy-keto and epoxy, were detected for the first time during the oxidation process of corn oil. This fact is of great interest since these oxylipins are associated with several diseases. Moreover, the formation of formic acid as well as poly-formates, poly-hydroxy and poly-ether groups was proved. Simultaneously, the monitoring of well-known oxylipins, also related with different degenerative diseases, was carried out.
Significance of Your Research to the AOCS Membership: The study has proved that 1H NMR is a very useful technique to follow lipid oxidation processes in-depth. The obtained data will provide new insights into the degradative process of edible oils and also of biological system, constituting valuable information for future studies in which lipid oxidation is involved.

**Quantitative Analysis of Esterified Oxylipin; Exploring Involvement in Neurodegenerative Disorders.** Yurika Otoki, Tohoku University, Japan
Polyunsaturated fatty acids (PUFA) are precursors to hundreds of bioactive lipid mediators known as oxylipins. PUFA can be converted into oxylipins enzymatically or non-enzymatically. In the brain, oxylipins regulate multiple biological processes, including angiogenesis, vasodilation, vasoconstriction, signaling, inflammation, and the resolution of inflammation. Recently, we reported that the majority of oxylipins in brain are esterified to complex lipids. The significance of this esterification process and its physiological function is not known due to the lack of an analytical method. In this presentation, an analytical method for separating oxylipins within phospholipids and neutral lipids (i.e., triacylglycerol and cholesteryl ester), and direct analysis of bound oxylipin will be discussed. The proposed method can be used to understand the potential role of esterified oxylipins in neurodegenerative disorders, and metabolic processes that regulate their turnover.

**Quantitative Spatiotemporal Mapping of Lipid and Protein Oxidation in Mayonnaise.** John Van Duynhoven, Unilever, Netherlands; Suyeon Yang, Wageningen University, Netherlands; Donny Merkx, Unilever, Wageningen University, Netherlands; Lia Verhoeff, Unilever, Netherlands; Johannes Hohlbein, Wageningen University, Netherlands
Lipid oxidation in mayonnaise is mediated by the spatial arrangement of catalytic active emulsifiers at the oil-water interface and colloidal transport of oxidation reaction intermediates. In order to unravel these mechanisms, a method is needed for assessing lipid oxidation in a time- and space-dependent manner. We monitored changes in both the fluorescence emission of a lipophilic dye BODIPY665/676 and protein autofluorescence to obtain spatiotemporal maps of local lipid and protein oxidation rates. Furthermore, we explored the used of fluorescent spintraps to localize radical formation. To map lipid and protein co-oxidation at the micron-scale over time, we implemented a sample carrier that is mounted on the objective of a confocal laser scanning microscope. Thus, the same volume of mayonnaise can be monitored over a shelf-life of weeks under defined environmental conditions. We quantified time-dependent features by first segmenting individual droplets and then tracked the oxidation of individual droplets. This approach was demonstrated on mayonnaise samples which differed in their levels of tocopherol and ascorbic acid, which are known to respectively act as oil- and water-soluble antioxidants. The observed spatially heterogeneous oxidation of proteins at the oil-water interface is in line with heterogeneous distribution of lipoprotein granules from the egg yolk used for emulsification. We observed that ascorbic acid acts as either lipid anti- or pro-oxidant depending on the presence of lipid soluble antioxidants. Protein radical formation occurs at early stage of oxidation and its spatial heterogeneity points to involvement of lipoprotein granules. Impact of droplet size on local lipid and protein oxidation rates was minor compared to the anti- and pro-oxidant effects, which can be attributed to inter-droplet exchange of oxidation intermediates. The presented results demonstrate that fluorescence imaging can be applied for unravelling the roles of colloidal structure and transport in mediating lipid oxidation in complex food emulsions.
In this work a method will be presented to map lipid and protein co-oxidation in mayonnaise at the microscopic scale. With this method direct insight can be obtained at local oxidation kinetics within separate droplets, at the droplet interface and in the water phase. This will be demonstrated by unravelling the roles of two well know pro- and anti-oxidants.

**Structural Analysis of Lipid Oxidation Products Using Mass Spectrometry.** Shunji Kato, Tohoku University, Japan
Lipid oxidation is involved in various biological phenomena (e.g., oxylipin generation and oxidative stress) and food quality (e.g., pleasant- and off-flavor). Of oxidized

lipid structures, the hydroperoxyl group position of lipid hydroperoxides (LOOH) is a critical factor to determine their roles. Such LOOH positional isomers (regioisomers) are generated by all or either of radical-, singlet oxygen- or enzymatic-oxidation which depends on the certain reactions that takes place in the samples. Importantly, the type of reaction that takes place reflects the regioisomeric structure of LOOH.

We previously developed mass spectrometric methods using cation-$\pi$ interaction for the highly selective and sensitive determination of LOOH regioisomers. The analysis using this method allowed us to analyze phospholipid hydroperoxide regioisomers in human plasma, which demonstrated that plasma phospholipid peroxidation is initiated by radical and/or enzymatic oxidation rather than singlet oxygen oxidation. We also applied this method to analyze vegetable oils (triacylglycerol) and revealed the oxidation mechanisms during industrial or culinary processes. This presentation will discuss about the methods and some of analyses.

## Continuing the Debate—Shelf Life vs. Accelerated Storage for Evaluating Antioxidant Effectiveness

*Chairs Natalie Oswell, Kalsec Inc., USA; J. David Pinkston, Archer Daniels Midland Company, USA*

**Accelerated Shelf-life Tests of Food Undergoing Oxidation: Uncertainties, Pitfalls and Perspectives.** Sonia Calligaris, University of Udine, Italy; Lara Manzocco, University of Udine, Italy; Maria Cristina Nicoli, University of Udine, Italy

Oxidation is the most common event leading to the end of shelf life of microbiologically stable foods with medium-long storage stability. The application of reliable shelf life assessment protocols is crucial to verify how long the product will last before it becomes oxidized to an unacceptable level for the consumers. Testing methodologies under actual and accelerated storage conditions can be applied to this aim. However, the possibility to speed up shelf life testing is highly demanded by food industry. Among all environmental factors that might be potentially exploited to accelerate food alterative events, temperature is certainly the most frequently used. Beside temperature, also light might be exploited. When properly applied, accelerating shelf life testing allows to definitively reduce the time needed to estimate product shelf life, accomplishing with industrial need. However, a number of threats could arise during the acceleration process due to the complexity of foods, leading to dangerous over- or underestimations of food shelf life.

This lecture will give an overview of the basic principles and procedures for the application of ASLT for the shelf life prediction of foods, with special emphasis on the application of temperature and light as accelerating factors. Uncertainties, pitfalls and future research needs on this topic will be critically discussed.

**Chemical Accuracy vs Expediency in [accelerated] Shelf-life Assays.** Karen Schaich, Rutgers University, USA

There has been a perpetual battle over measurements of shelf life. Is it better to use standard ambient condition shelf-life testing that presents an accurate picture the timing and pattern of oxidative degradation in a given food but requires long times, or can accelerated testing using high temperature storage give answers faster and be mathematically manipulated to predict normal shelf life? Arguments presented for accelerated shelf-life approaches are that rapid testing is need to expeditiously track effects of formulation and processing changes and that elevated temperatures merely speed up lipid oxidation according to the Arrhenius equation but do not change products. However, lipid oxidation pathways, reaction activation energies, and products change dramatically with temperature. What is measured reflects a competition between increased formation of products and often even faster transformation to unidentified downstream products or degradation to small products that get lost in analyses. Thus, high temperature studies can often result in paradoxically lower product levels that are not recognized when single products are analyzed and one storage temperature is used. Such data underestimates the extent of lipid oxidation and its extrapolation to lower temperatures overestimates useful shelf life. How can the needs for accuracy and expediency in shelf life studies be reconciled? This paper takes a fresh look at effects of temperature on lipid oxidation and the total process of oxidative degradation in foods then relates these to analytical procedures required to provide accurate pictures of important changes affecting quality and safety plus a rational basis for new approaches to shelf life measurements.

**Use of Oxygen Bomb Methods for Accelerated Shelf-Life Measurements.** Shiekha Mittal, Rutgers University, USA; Karen Schaich, Rutgers University, USA

Measurement of oxygen consumption by commercial oxygen bombs has been used as a simple accelerated shelf life analysis to compare effectiveness of formulations and processes. However, mismatch between induction periods reported by oxygen consumption and other methods have raised questions about what is being measured in the different tests and which are most accurate. To follow the chemistry occurring in one oxygen bomb in common use, commercial vegetable oil was heated in sealed cells over a range of oxygen pressures and temperatures until past the onset of active oxygen consumption. Oil samples were withdrawn and analyzed for conjugated dienes, hydroperoxides, epoxides, and carbonyls throughout this period.

Results show that especially at lower temperatures and oxygen pressures, active formation and accumulation of products proceeds before apparent onset of oxygen consumption. This timing difference decreases as temperature and oxygen pressure increase. The paper proposes instrumental and chemical explanations for this product disparity, along with appropriate conditions for obtaining synchronized chemistry with the instrumentation. It also discusses how to reconcile oxygen consumption results with other product analyses in accelerated shelf life studies.

**When Analytical Tools Solve Our Needs: Monitoring and Predicting Shelf Life.** David Bolliet, Kalsec Inc. USA

Of the many different analytical means of monitoring the shelf life of food products, some techniques are more useful than others. When trying to ultimately predict the quality and the safety of food for the consumer, it is essential to use the right analytical tools. Those can range from the very simple (temperature monitoring, chemical testing), the now mostly mainstream spectroscopic and chromatographic options, or the development of machine learning models. We will review some of the true and tested analytical tools, as well as some of the more disruptive solutions that are being implemented to help predict shelf life.

## Industry Updates I

*Chair Ryan Dietzenbach, CPM Roskamp Champion, USA*

**Adsorptive Removal of Contaminants from Fats and Oils for Production of Biofuels.** Brian Cooke, Clariant, USA

Both Biodiesel and Renewable Diesel are derived from fats and oils. These fats and oils can often present challenges in the process due to contaminants that interfere with the efficiency of the reaction often resulting in a decrease of catalyst activity. This study looks at the removal of these contaminants to help with the biodiesel reaction efficiencies as well as helping to protect the catalyst used during the hydrotreating process for renewable diesel. The use of activated clay has been shown to remove these contaminants through the process of adsorption.

The life of the catalyst for renewable diesel can be significantly improved by removing contaminants such as metals and phosphorus. This can result in significant cost savings during the production of renewable diesel.

The activated clay can also be used to remove contaminants from the crude biodiesel, such as glycerol, soap and metals. It is imperative that these contaminants are removed to ensure that the biodiesel will meet the desired specifications.

**Detection of Organophosphate Pesticides in Olive Oil with Metrohm Instant SERS Analyzer (Misa).** Wei Yu, Metrohm, USA

Organophosphate pesticides are widely used in olive cultivation for the control of insect pests. Due to the high solubility of these pesticides in oils and the minimal processing of virgin oils, these harmful chemicals may accumulate in the end product. Detection of pesticide residues typically require the use of analytical techniques such as GC-MS and HPLC, but technical complexity and cost requirements limits their usage to the confines of well-equipped laboratories.

The Metrohm Instant SERS Analyzer (Misa) from Metrohm is a portable Raman spectrometer for performing Surface Enhanced Raman Scattering (SERS) measurements. SERS is an extension of Raman spectroscopy which allow detection and identification of analytes in concentrations as low as parts per billion. Misa was developed with the focus for a simple, rapid and green testing solution to address food safety threats.

In this study, we demonstrate the use of Misa in detecting Fenthion in extra virgin olive oil (EVOO). Fenthion is an organophosphate that has been classified as a restricted-use insecticide by the US EPA. However, the use of this pesticide in olive orchards in Mediterranean countries results in EVOO that occasionally exceed the maximum residue limits (1mg/kg) established for virgin olive oil. Misa easily achieves sensitive trace detection of Fenthion in spiked EVOO after a simple organic solvent extraction in ~15 minutes. The assay is based on the acquisition of SERS-specific spectra for Fenthion in the extract using Misa with a gold colloid solution.

Misa features simple one-touch operation and guided workflows for many common pesticides and contaminants in a variety of food matrices, making it a valuable tool for the rapid detection and identification of trace food contaminants.

**EcoXtract®: A Bio-based and Clean Alternative to Hexane in Oil Extraction.** Gabriel DUFOUR, EcoXtract (Pennakem Europa), France

**EcoXtract®** offers a sustainable way to process oilseeds and extract plant-based ingredients, oils and proteins.

**EcoXtract®** is an innovative technological solution (>99.9% of **2-methyloxolane)**:

**1. Nature-based solution** - EcoXtract® production is derived from hemicellulose, found in many agricultural by-products

**2. High performance** in oil extraction (similar extraction yield compared to hexane

**3. Safe toxicological profile** for workers and consumers - scientifically backed by more than 12 years of data collection

**4. Environmental-friendly** - no harmful impact on environment

**Exploring the structure-function relationships of protein based surfactants for food emulsions and foams.** Jeffrey Sanders, Schrodinger, USA
As consumer trends move towards healthier foods with natural ingredients, biopolymers like proteins, carbohydrates, and lipids are gaining popularity in food products. Functioning as emulsifiers, thickening agents, coatings and encapsulation materials, self-assembling proteins are excellent candidates for novel food formulations. Protein structure/function is dependent on physicochemical properties of their environment and understanding this relationship is critical for candidate selection and downstream processing properties. Decades of computational chemistry research has expanded our knowledge of protein structure and function, providing insight that is either inaccessible via experimental methods or would require expensive time-consuming experimentation. Using high throughput physics-based simulations methods, we will explore the structural properties of hydrophobins, a protein-based surfactant made by filamentous fungi. In addition, multi-scale simulations of hydrophobin mixtures are performed to simulate the self-assembly process. This information is necessary not only for selection of protein sources, but also engineering of natural products for specific food applications.

**Optimizing Flaking Mill Performance in Oilseed Applications.** Doug Reuscher, Roskamp Champion, USA
Optimizing flaking mill efficiency in oilseed applications is the focus of this presentation. I will cover seed preparation, maintenance, and capacity of the flaking mill to identify opportunities for improvement. Manufacturing uniform product is critical for efficient operation. Flaking mill optimization starts with supplying properly conditioned & cracked seed to the mill. I will explain different dehulling systems used to prepare the seed for flaking. Then I will discuss key maintenance areas of the flaking mill. These areas include proper feeding and adjustments needed to ensure the seed does not bypass the rolls. I will finish with the importance of roll grinding along with frequency, duration, and roll type.

## Industry Updates III

*Chair Bruce Patsey, Oil-Dri Corp., USA*
**Improvement of Plant Protein Functionality by Protein Glutaminase.** Keita Okuda, Amano Enzyme USA, USA; Shotaro Yamaguchi, Amano Enzyme Inc., Japan; Hiroki Fujioka, Amano Enzyme Europe, USA
The industry is facing the challenge of low solubility of plant proteins. Protein Glutaminase "Amano" 500™ (PG500) is the first food enzyme to improve the solubility of proteins at low pH levels. PG500 catalyzes the deamidation of the amino group of glutamine residues in the protein sequence, so that it improves the hydrophilic characteristic of the protein.
When pea protein isolate was treated with PG500 at 50 °C for 1 hour, it was observed that the solubility was improved by 20% at neutral pH and the isoelectric point shifted to pH5.5 from pH6.0. It was confirmed that PG500 worked especially well with popular plant-based proteins. As a deliverable, it was also found that PG500 treated plant-based milk was not curdled in coffee. In addition, tribology analysis showed that PG500 treatment reduced the coefficient of friction of plant-based milks by about 25%, suggesting that PG500 treated plant-based milk has a milder mouthfeel.
Moreover, when PG500 was applied to the early stage in the pea protein isolating process, the yield increased by 15% in a bench scale and the resulting pea protein isolate possessed the benefits PG500 brings.
These results indicate that PG500 is able to give greater performance in the plant-based application.

**Isolation of Vitamin A, E, D and Cholesterol from Food and Feed Using an Automated Method.** Marleen van Aardt, ANKOM Technology, USA
An effective method is presented for the simultaneous and automatic isolation of vitamin A, E, D and cholesterol from food and feed. Multiple samples are automatically saponified, extracted, and the solvent is evaporated within 2½ hours, while eliminating technician involvement. Subsequent quantitation could include HPLC-DAD/FLD, HPLC-MS-MS, or GD-FID. Method validation includes (i) trueness measurement using certified reference materials (NIST 1869 - Infant Formula, NIST 1546 – Meat Homogenate), (ii) trueness measurement as it relates to matrix spikes, (iii) precision assessment using high fat/high protein and fortified lab control samples (Sockeye Salmon Jerky, Infant Formula), and (iv) robustness testing using Youden statistics. This automated method employs SPE technology as extraction technique that significantly reduces solvent usage and eliminates complications such as emulsions, which are typically associated with liquid-liquid extraction. The efficiency of clean-up is reflected in clear extracts and chromatograms that compare well with industry standard methods. Emphasis was placed on validating the method's capability to fully isolate target analytes from a variety of food and feed samples from all nine sectors of the AOAC food triangle.

## Analytical Poster Session

*Poster Chair Ali Reza Fardin-Kia, US Food and Drug Administration, USA*
**A rapid and efficient method for dialkyl ketones and sterols determination in fat.** Steven Mascrez, Gembloux Agro Bio Tech, University of Liège, Belgium;

Sabine Danthine, University of Liège, Belgium; Giorgia Purcaro, University of Liège, Belgium

The present work deals with the optimization of a fast method for the determination of dialkyl ketones (DAKs) in interesterified fats. Microwave-assisted saponification was optimized, followed by purification on a lab-packed silica solid-phase extraction cartridge. The method proposed may be used for the determination of DAKs or both DAKs and sterols by simply eluting an additional fraction from the solid-phase cartridge. The final determination was performed by gas chromatography- flame ionization detector (GC-FID) for quantification and gas chromatography-mass spectrometry (GC-MS) for confirmation purposes. The proposed method showed good recoveries ( >80%) and limit of quantification (0.04–0.07 μg/g for the 4 standard DAKs and of 0.07 μg/g for α-cholestanol). Repeatabilities (n=3) were below 15% for DAKs and generally lower than 6% for sterols. Accuracy on the entire sterol profile was confirmed in comparison to the International Olive Council reference method. The method was finally applied to real-world samples before and after chemical interesterification

**A Summary of the Research Activities at the U.S. FDA Related to the Analysis and Occurrence of MCPD Esters and Glycidyl Esters in Edible Oils, Infant Formula, and Other Processed Foods.** Jessica Beekman, US Food and Drug Admin, USA; Michael Granvogl, University of Hohenheim, Germany; Shaun MacMahon, U.S. Food and Drug Administration, USA

3-Monochloro-1,2-propanediol (3-MCPD) esters, 2-monochloro-1,3-propanediol (2-MCPD) esters, and glycidyl esters are process-induced chemical contaminants formed during the deodorization step of the refining of edible oils. These compounds are considered potentially carcinogenic and/or genotoxic, making their presence in refined oils and other processed foods containing these oils a potential health concern. Over the last 10 years, researchers at the U.S. Food and Drug Administration (FDA) have developed methods for the analysis of these contaminants in refined vegetable oils, infant formula, and other complex food matrices in an effort to determine their occurrence in many food products on the U.S. market and abroad. This poster will summarize the extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies developed at the U.S. FDA. In addition, the occurrence of these compounds in a variety of foods (including infant formula) and the impact of food processing on the concentrations of these compounds in the final products will be presented.

**Development of an Isotope-Dilution UHPLC-QqQ-MS/MS-Based Method for Simultaneous Determination of Typical Advanced Glycation End-Products and Acrylamide in Baked and Fried Foods.** Weiwei Cheng, Shenzhen University, China (People's Republic); Guoqin Liu, South China University of Technology, China (People's Republic); Ka-Wing, Cheng, Shenzhen University, Hong Kong

Nε-carboxymethyllysine (CML), Nε-carboxyethyllysine (CEL), and acrylamide (AA) are Maillard reaction-derived hazardous substances, which are threatening human health and life through various consumption of food stuff containing these trace compounds. In this work, a stable isotope dilution ultra-high-performance liquid chromatography triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS) method was developed and validated for simultaneous determination of CML, CEL, and AA in baked and fried foods. Ground food samples were extracted with acetone followed by two parallel assays. In Assay A, a cleanup procedure based on dispersive solid-phase extraction was conducted for AA, free CML and CEL analysis using the supernatant; In Assay B, a multi-step process including reduction, protein precipitation, acid hydrolysis, and solid-phase extraction was conducted for bound CML and CEL analysis using the precipitation. The developed method was validated in terms of linearity, sensitivity (limit of detection, LOD; limit of quantitation, LOQ), accuracy, and precision. The results showed that the method had a wide linear range (0.25–500 ng/mL for CML and CEL, 0.5–500 ng/mL for AA), low LOD and LOQ (0.47–0.94 and 1.52–1.91 μg/kg, respectively), and good linearity (R2>0.999). The recovery test on baby biscuit and french fries samples showed the recovery rates of 90.2–108.3% for CML, 89.0–106.1% for CEL, and 94.5–112.3% for AA with satisfactory precision (RSD< 10%). Finally, the developed method was successfully applied to eleven baked and fried food samples, and total CML, CEL, and AA contents varied in the range of 4.07–35.88 mg/kg, 1.99–14.49 mg/kg, and 5.56–506.64 μg/kg, respectively. Therefore, the isotope dilution UHPLC-QqQ-MS/MS method developed herein is promising for routine analysis of CML, CEL, and AA in baked and fried foods.

**Differentiating Virgin Coconut Oil from Refined, Bleached, Deodorized Coconut Oil by Untargeted 13C NMR Profiling and Unsupervised Univariate and Multivariate Statistical Techniques.** Fabian Dayrit, Ateneo De Manila University, Philippines; Mark Joseph Garrovillas, Ateneo de Manila University, Philippines; Lolita Lagurin, Ateneo de Manila University, Philippines

Virgin coconut oil (VCO) is a high value functional food that is obtained from the fresh, mature kernel of the coconut by mechanical or natural means, without the use of chemicals or high heat. VCO is produced using any of three processes—centrifuge, expeller, and natural fermentation. On the other hand, refined, bleached, deodorized coconut oil (RBDCO) is produced from

copra and requires processing with chemicals and high heat. Because of their similarity in composition, it is difficult to differentiate them using physico-chemical analysis. This study used 13C NMR spectroscopy and a metabolomic approach to differentiate VCO (n=57) from RBDCO (n=21) samples. After routine 13C NMR processing, the chemical shift data were aligned, binned and normalized. The 13C chemical shifts were analyzed using volcano plot [8] to identify the statistically significant chemical shifts responsible for differentiating VCO and RBDCO. This significantly improved the discrimination of VCO and RBDCO as shown by Principal Component Analysis. This will be helpful in creating better and more optimized models.

**Fast screening of vitamin E in dietary supplements by thin layer chromatography.** Walter Vetter, University of Hohenheim, Germany; Sina Schweizer, University of Hohenheim, Germany; Alexander Kröpfl, University of Hohenheim, Germany

Vitamin E is the summarizing term for a number of fat-soluble vitamins which feature a chromanol ring which is substituted with an isoprenoic side chain. Classic members of the vitamin E family are a-, β-, g-, and d-tocopherol (no double bond in the isoprenoic side chain) and the corresponding tocotrienols (three double bonds). Vitamin E is an important antioxidant which can suppress lipid autoxidation.

For a fast screening of capsules of vitamin E containing dietary supplements, contents were mixed with acidified water and vitamin E compounds were extracted with nhexane. After addition of NaCl solution (saturated), samples were centrifuged. Aliquots of the hexane phase were developed on silica 60 plates with fluorescence indicator using n-hexane/ethyl acetate/acetic acid (90/10/2, v/v/v) as the mobile phase. This system enabled the full separation of the eight vitamin E compounds. a-Tocomonoenol, a relevant minor vitamin E compound of palm oil, could be separated as well. Separated vitamin E compounds were quantified after derivatisation (sulphuric acid/methanol) by densitometry using a TLC scanner using the UV absorption of 293 nm. LOD and LOQ were calculated according to DIN 32645 reaching from 0.12 μg for g-tocopherol to 0.37 μg for d-tocopherol. Contents detected in commercial vitamin E dietary supplement capsules reached up to 250 mg total vitamin E per capsule.

**FT-NIR evaluation for the quantification of fatty acids in fats and oils.** Pierre-Alain Golay, Nestle Research, Switzerland; Marco Scacchi, SOFINOL SA, Switzerland; Morena, Bonato, SOFINOL SA; Switzerland

Important progresses have been made these last years in the development of green analytical technologies addressing safety and environmental aspects.

Vibration technologies are an example of green technologies, offering several solutions available as benchtop, portable and hand pocket instruments, which can be easily used and installed in environments different than laboratory ones.

In order to reduce the use of organic solvents and saving time and costs during analysis, Fourier Transfer Near Infrared (FT-NIR) was evaluated in terms of trueness and precision for the quantification of fatty acids in fats and oils.

Several oil mixes containing various proportions of different oils (vegetable and marine) were selected and fatty acids were quantified by gas chromatography coupled with flame ionization detector (GC-FID) after alkaline hydrolysis of triacylglycerols and methylation of released fatty acids and then analyzed on a Tango FT-NIR (Bruker®).

FT-NIR results precision were highly influenced by the calibration models (often to broad), provided with the instrument, however when calibration model was adjusted using true and accurate fatty acid values (g/100g), results for individual fatty acids were comparable (< 10%) to those obtained with chromatographic methods. Best method similarity scores (< 5%) were obtained for sums of fatty acids (i.e., SFA, MUFA, PUFA, w-3, w-6 & w-9) and overall repeatability values were found comparable between GC-FID and FT-NIR, correlation (R2) exceed 0.99.

Therefore, when proper FT-NIR calibration models are adapted (considering specificity of oils, but also purity/refining degree), FT-NIR can be used to quantify fatty acids in g/100g in fats and oils within the limits of compliance. However, further efforts need to be made for FT-NIR calibration maintenance and more friendly software solution.

**GC/MS identification of alkylresorcinols after fractionation using all-liquid chromatographic methods.** Walter Vetter, University of Hohenheim, Germany; Tim Hammerschick, University of Hohenheim, Germany; Tim Wagner, University of Hohenheim, Germany; Lukas Wallinger, University of Hohenheim, Germany

Alkylresorcinols (5-alkyl-1,3-dihydroxybenzenes, ARs) are bioactive phenolic lipid compounds which are abundant in cereals. The backbone of ARs is a 1,3-dihydroxybenzene (resorcinol) with a (un)saturated hydrocarbon chain of 15 to 27 C atoms, which are found in cereals, at position C-5. In addition to the highest content of ARs, rye also has a large variety of AR structures. For example, keto groups can be present as substituents of the hydrocarbon chain. Remarkably, a high number of beneficial biological activities are attributed to ARs.

ARs of cereals were extracted with the azeotropic mixture cyclohexane/ethyl acetate (46/54, w/w). AR profiles were determined by means of preparative all-liquid chromatography techniques, i.e., countercurrent chromatography (CCC) and the centrifugal partition chromatography (CPC). After CPC and CCC fractionation

using the biphasic solvent system nhexane/ethyl acetate/methanol/water (9/1/9/1, v/v/v/v), AR profiles and structures were investigated by GC/MS(-SIM) analysis after silylation, acetylation or formation of DMDS adducts.
This combining method enabled the detection of 74 different ARs, including several novel ARs (even-numbered monounsaturated ARs, triunsaturated AR, odd-numbered methylated ARs and saturated oxo-AR). Using DMDS adducts, the positions of the double bonds of monounsaturated ARs and oxo-ARs were determined in n-9-, n-7- and n-5-position.
The presented method of combining CCC fractionation with a sensitive detection method increased the number of detectable ARs and can also be applied to other substance classes. Especially the preparative character and the orthogonal separation properties of CCC make this technique a very useful tool in analytics.

**Identification of degraded oxidized fatty acids in heated soybean oil by electrospray ionization multistage mass spectrometry (ESI-MSn).** Xu Li, Jiangnan University, China (People's Republic); Donghao Wang, University of Texas at Austin, USA; Zhen Wang, University of Texas at Austin, China (People's Republic); Xingguo Wang, Jiangnan University, China (People's Republic); J. Thomas Brenna, University of Texas at Austin, USA
Identification of oxidation products in oxidized and abused oils is increasingly recognized as important because of known health risks for some products, and because known standards are seldom available commercially. Here we designed a scheme for the mass spectrometric analysis of degraded oxidized fatty acids using conventional (50% linoleic acid) soy oil. Oil was oxidized in air and analyzed by direct infusion into an LTQ linear ion trap of nominal mass resolution and operated in MS2 or MS3 modes. Characteristic fragments that included carboxylic group were first identified. Fragmentation rules of hydroperoxy-, oxo-, hydroxy-, epoxy- fatty acids were systematically developed based on 41 normal fatty acids and 15 oxidized fatty acids. As expected, MS2 and MS3 were not sufficient to identify the position of double bond. Oxidized products from C18:1, C18:2 and C18:3 based on free radical mechanisms were then assembled into a search list of candidate structures. Finally, several oxidation products from heated soybean oil, oxidized fatty acids were identified according to the scheme. This interpretation system is expected to be of utility in manual and possibly automated interpretation of oxidation products.

**Multiple Light Scattering for Quantitative Stability Analysis of a Double Emulsion.** Gordon Irvine, Formulaction Inc, USA; Christelle Tisserand, Formulaction Inc, France; Mathias Fleury, Formulaction Inc, France; Yoann Lefeuvre, Formulaction Inc, France; Pascal Bru, Formulaction Inc, France; Gordon Irvine, Formulaction Inc, USA; Charles Nider, USA; and Gerard Meunier, Formulaction Inc, France.
The development of stable emulsions is critical for formulation chemists who desire a stable, well-dispersed product with an acceptable shelf-life. New materials and regulations require chemists to develop new formulas to meet these criteria. Visual analysis of these emulsions can be time-consuming and subjective, causing project delays and a dearth of qualitative results.
Herein, we present a technique based on Static Multiple Light Scattering (SMLS) to quantitatively examine stability, investigate destabilization mechanisms, and rapidly determine shelf life. It has proven to be a useful technique to characterize the dispersion state of colloidal samples and the mean diameter of particles in concentrated dispersions up to 95% volume fraction. Where other optical techniques require extreme dilution and risk denaturing the sample, SMLS allows for one-click analysis, without sample preparation or dilution, even for concentrated suspensions. Different instability phenomena such as flocculation, coalescence, sedimentation, clarification and creaming can be measured, quantified and predicted with great accuracy. Such phenomena can be observed up to 200 times quicker than the naked eye and long-term studies of stability and shelf-life can be easily performed to rapidly optimize critical formulations.
In a representative example, we analyzed double emulsions which are colloidal systems of great interest for the cosmetics industry as they enable encapsulation of both hydrophilic and lipophilic molecules. The release of an encapsulated active in the internal emulsion was studied using SMLS technology at various temperatures and surfactant concentrations. We were able to simulate topical applications and obtained detailed stability data with a mere 30-minute experiment.

**Multiple-cumulative trapping non-saturated headspace SPME: A powerful tool to increase volatile fingerprints.** Steven Mascrez, Gembloux Agro Bio Tech, University of Liège, Belgium; Giorgia Purcaro, University of Liège, Belgium
Headspace solid-phase microextraction (HS-SPME) is a solvent-free sample preparation method widely applied in many fields of applications. The theory that underlays this extraction technique is based on the equilibrium among a three-phase system, i.e., sample-headspace-fiber. A compromise between sensitivity and extraction time is usually needed to optimize the sample throughput, mainly when a large number of samples are analyzed, as usually the case in cross-samples studies. This work explores the capability of multiple-cumulative trapping solid-phase microextraction on the characterization of the aroma profiling

of olive oils, exploiting the automation capability of a novel headspace autosampler. It was shown that multiple-cumulative solid-phase microextraction has the potential to improve the overall sensitivity and burst the level of information for cross-sample studies by using cumulative shorter extraction times. Moreover, contrary to what is usually applied, the selection of the sample volume which does not saturate the headspace (i.e., even 10 times lower than the commonly used quantity), is of high relevance in order to maximize the information extractable.

In this work, the fingerprinting of olive oil is explored to discriminate among extra virgin, virgin, and lampante oil, a challenging task of interest for quality and authenticity assessment. The different conditions were compared, considering the general information as pattern analysis, rather than intensity-wise. The best results were obtained by using 0.1 g of samples, rather than the typical 1.5 g (or even more) found in the literature. Moreover, the use of multiple-cumulative extraction allows for better discrimination among the different groups compared to a single extraction. Finally, the increased separation power obtained by two-dimensional comprehensive gas chromatography (GC$\times$GC) was exploited to enhance further the identification capability.

**Off-flavor Research & Application in Cereal & Oil Food with Different Extraction and Identification Methods.** Jing Wang, Wilmar International, China (People's Republic)

In recent years, the off-flavor problem of cereal & oil food is prominent. The economic loss caused by food corruption is up to hundreds of billion yuan every year, of which the loss of cereal & oil food accounts for 15% in the normal temperature circulation process, second only to the proportion of vegetables and fruits. Based on the off-flavor problem of cereal & oil food, this paper mainly elaborates the off-flavor source, influence factors, analysis method, application practice, elimination and prospects. In terms of practical application, some typical off-flavor description is defined in cereal & oil food, such as "beany/grass" in peas, "stale/musty" in grains, "fusty/musty" in olive oil, "fishy" in soybean oil, "rancid" in vegetable oil, etc. They are mainly generated through fatty acid oxidative rancidity, thermal treatment, microbial metabolism and environmental pollution. The key off-flavor compounds identified is often combined with multiple detection indexes, such as microorganisms, fatty acids and other indexes for sources predicted, which is the characteristic of off-flavor analysis. The ways of eliminating the off- flavor in cereal & oil food including microcapsule, adding antioxidants, defatting, thermal processing, $\gamma$ irradiation and mask, etc. In conclusion, research into the typical off- flavor in cereal & oil food requires further study.

**Simultaneous determination of deoxynivalenol and deoxynivalenol-3-glucoside in wheat and its products by HPLC-UV with immunoaffinity cleanup.** Hong Yang, Wilmar Intl, China (People's Republic); Wen-Ming Cao, Wilmar Intl, China (People's Republic); Xue-Qing Wei, Wilmar Intl, China (People's Republic); Xuebing Xu, Wilmar Intl, China (People's Republic)

Major masked deoxynivalenol, deoxynivalenol-3-glucoside, widely present in wheat and its products. It is a potential risk of being hydrolyzed and converted to DON during the digestive process of mammals. A simple and sensitive method for the simultaneous determination of deoxynivalenol and deoxynivalenol-3-glucoside was validated in the present study. The immunoaffinity cleanup combined with high-performance liquid chromatography - ultraviolet detector (HPLC-UV) was applied. The method was validated as follows: linearity (R2 > 0.99) curves were established in the range of 100–2000 ug/kg; recovery rates ranged from 84.5 to 100.7% for all the analytes; good precision (relative standard deviation < 4.90%), and adequate quantitation limits (100 ug/kg for deoxynivalenol-3-glucoside and 200 ug/kg for deoxynivalenol) were achieved, respectively. The validated method was successfully applied to the analysis of wheat and its products.

**The AOCS analytical method process: how AOCS Official Methods are produced.** Scott Bloomer, American Oil Chemists' Society, USA; Fiona Liu, AOCS, USA; Xin Wu, AOCS, USA

Analytical methods are not considered "official" until they have been subjected to review by experts and tested in collaborative trials. The AOCS method development process enjoys a worldwide reputation for efficiency. Rigorous scrutiny by AOCS analytical committees precedes and follows our collaborative trials. Final inspection by the AOCS Uniform Methods Committee (composed of veteran analytical scientists) ensures that new AOCS Official methods are well-written and suitable for use around the world.

***Abstracts for the following Analytical presentation were not provided:***

**Statistical Treatment of Proficiency Testing Data—How to Interpret Laboratory Proficiency Reports.** Xin Wu, AOCS, USA

**Desmet Ballestra—Science Behind Technology.** Orayne Mullings, Desmet Ballestra, USA

**Quality Control of Edible Oils with Advanced NIR Technology.** Mary Landis, FOSS North America

**Comparative Analytical Tools for Predicting Shelf-lifes: Do They Work?** Richard Della Porta, Pepsico/Frito-Lay, USA

**Oil-Dri Bleaching Earth Products: Meeting Customers' Demands for Quality Products and Technical.** Bruce Patsey, Oil-Dri, Corp., USA

## Biotechnology Division

*Vice Chair: Todd Underiner, Procter & Gamble, USA*

## Biotechnology Division Student ePoster Pitch Competition

*Judges: Douglas Hayes, University of Tennessee, USA; Sarah Willet, Kerry, USA; Suzana Ferreira-Dias, University of Lisbon, Portugal; and Pierre Villeneuve, CIRAD Service Comptable, France*

Sponsored by the AOCS Foundation and supported by the AOCS Biotechnology Division

**Application of co-cultures of fungal mycelium during solid-state fermentation of canola meal for potential feed application.** Ahmad Alhomodi, South Dakota State University, USA; Andrea Zavadil, South Dakota State University, USA; Mark Berhow, USDA ARS NCAUR, USA; William Gibbons, South Dakota State University, USA; Bishnu Karki, South Dakota State University, USA

Canola meal (CM) is a by-product of canola seed oil extraction and contains up to 50% protein on a dry basis. Despite CM being the high protein commodity with well-balanced amino acid composition, it remains one of the more underutilized by-products of the oil processing industry. Only a fraction of produced CM goes into animal feeds as presence of antinutrients such as phenolics, glucosinolates (GLS), phytates, and high fiber content makes this product undesirable. Mixed microbial cultures have been used successfully in commercial scale, fermentations such as in wastewater treatment plants, for soil remediation, in biogas production, and in the production of traditional foods such as cheese, yoghurt, pickles and in alcoholic beverage production. Thus, in this study, the mono, bi, and tri-cultivation of Aureobasidium pullulans, Neurospora crassa and Trichoderma reesei in solid state fermentation were applied to improve the nutritional value of hexane extracted canola meal along with the reduction of antinational factors for animal feed applications. The data showed that fungal cultivation had positive effects on the level of protein, fiber and, glucosinolates (GLS). Monoculture of N. crassa exhibited the highest protein level of 49%. The combination of A. pullulans and N. crassa provided the highest reduction of crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) by 21.9%, 1.7% and 9.1% respectively. Bi-culture of A. pullulans and T. reesei resulted in the best GLS reduction by 81.3%. These results indicate that each fungal strain owns different enzymatic ability and selectively can work with other fungi in synergistic relationship for better fungal conversion of canola meal.

**Impacts of discoloration on the antibacterial and antifungal bioactivities of a porcine cruor hydrolysate.** Aurore Cournoyer, Laval University, Canada; Zain Sanchez Reinoso, Laval University, Canada; Laila Ben Said, Laval University, Canada; Jacinthe Thibodeau, Laval University, Canada; Sergey Mikhaylin, Laval University, Canada; Ismail Fliss, Laval University, Canada; Laurent Bazinet, Laval University, Canada

Porcine blood is a major by-product from slaughterhouses and has a high potential for valorization. Indeed, the solid part of blood, designated as cruor, has a high proportion of proteins: 90 % of hemoglobin. By conducting enzymatic hydrolysis of porcine hemoglobin, using pepsin, a wide variety of peptides can be obtained. They are known to include bioactive peptides, such as antimicrobials. Many studies have been performed on hydrolysates from bovine hemoglobin but few on porcine hemoglobin. The color of the cruor caused by the presence of haem, can be removed by a discoloration procedure, where haem is precipitated. This step increases its potential for applications in food and more specifically white meat. The consequences of this additional step were evaluated on the antibacterial and antifungal activities. The results showed that, after precipitation of haem, the recovered peptides in raw hydrolysate and discolored one were different. Some peptides disappeared during this step. In addition, both hydrolysates showed antifungal activities but no antibacterial activity. The absence of antibacterial activity was due to the short duration of hydrolysis and the concentration of peptides. The degree of hydrolysis increases with time and, therefore, the length and concentration of peptides differ. However, in terms of antifungal activities, significant differences were observed between the two hydrolysates, along with the discoloration pellet. The raw hydrolysate had an antifungal activity up to 10 times higher than with the discolored one. The discoloration causes the loss and reduction of specific peptides, also found in the bioactive pellet. These precipitated peptides, which sequences were identified, would be responsible for the antifungal activity. To our knowledge, it is the first time that sequences of antifungal peptides from porcine cruor hydrolysate were proposed. The next step is the synthesis of these potential active peptides and to test them individually or in mixture.

**Tailoring Camelina sativa seed fatty acid composition via Fast Neutron mutagenesis.** Xinjie Liu, University of Saskatchewan, Canada; Helen Lui, Agriculture and Agri-Food Canada, Canada; Dan Hupka, Agriculture and Agri-Food Canada, Canada; Xiao Qui, University of Saskatchewan, Canada; Mark A. Smith, University of Saskatchewan, Agriculture and Agri-Food Canada, Canada

The oilseed crop Camelina sativa produces a seed oil rich in polyunsaturated fatty acids and natural antioxidants. The high protein meal from seed pressing is well suited for use in livestock feeds. As a relatively new crop, there is still considerable potential for camelina to be improved in agronomics, yield, oil quality and oil content. A potential obstacle to camelina improvement is the limited genetic diversity of the species. In this project, Fast Neutron (FN) induced mutagenesis was used to generate genetic variability, producing a range of mutations such as deletions, point mutations and rearrangement with the potential to disrupt tandemly duplicated genes in the camelina genome. The dose of irradiation used ranged from 7 to 49 Grey (Gy). Germination assays revealed that the germination rate of M1 seeds from all treatment doses was similar to that of the control seeds. Phenotypic mutations included plants with reduced branching, variable leaf shape and male sterility. Gas chromatography (GC) analysis of seed acyl composition identified a mutant line with 18:3 content close to 45% and a mutant line with 20:1 content reduced to 10%, as compared to 33% and 13% respectively in Camelina commercial cultivars. The next step is to determine the genotype of those mutant lines. The eventual goal of the project is to introduce new traits into elite cultivars through backcrossing and to develop a resource that can be made available to the camelina research community.

## Emerging Biotechnological Developments in Lipids

*Chairs Nuanyi Liang, University of California, Davis USA; Yomi Watanabe, Osaka Research Institute of Industrial Science & Technology, Japan*

**Antifungal Hydroxy Fatty Acids: Sources and Applications in Food and Agriculture.** Michael Gaenzle, University of Alberta, Canada; Nuanyi Liang, University of California, Davis, USA

Hydroxy fatty acids are present in some plant seed oils and produced by microbial or enzymatic transformation of fatty acids. Lipoxygenases convert linoleic acid to peroxides, which are reduced to the hydroxy fatty acids in the presence of thiol groups. Microbial conversion of fatty acids by hydratases also forms hydroxy fatty acids; this conversion is catalysed by lactic acid bacteria including food fermenting and plant associated lactobacilli. Plants that accumulate a substantial proportion of hydroxy fatty acids in the seed oil include castor beans (Ricinus communis L.), thyme (Thymus vulgaris L.), and Coriaria nepalensis Wall.

Hydroxy-fatty acid have antifungal activity which is dependent on the presence of one or more double bonds and the location of the hydroxyl groups. Unsaturated C18 fatty acids that carry the hydroxyl group at position 9 – 13 of the carbon chain inhibit mycelial fungi at concentrations exceeding 0.2 g / L while the MIC of unsaturated C18 fatty acids with the hydroxyl group at position 2 or 18 is 3–10 times higher (1). The activity of hydroxy fatty acids against phytopathogens; yeasts are generally resistant (1,2).

Hydroxy fatty acids are produced by lactobacilli during growth in sourdough fermentation and, together with other antifungal metabolites, inhibit growth of spoilage molds on bread (3,4). Hydroxy C18 fatty acids also inhibited phytopathogenic fungi on wheat and barley (2). Remarkably, the reduction of the disease severity was unrelated to the antifungal activity and thus likely involved stimulation of plant defense mechanisms (2).

In conclusion, the use of antifungal hydroxy fatty acids can complement or substitute current methods for plant protection and food preservation, however, the efficacy is highly dependent on the specific application.

1) Food Res Intern 134:09237; 2) Can J Plant Sci doi:10.1139/cjps-2020-0113. 3) Int J Food Microbiol 302:8. 4) Appl Environ Microbiol 79:1866.

**Documenting the Versatility and Robustness of Applying Phospholipases in Alkaline Refining.** Per Nielsen, Novozymes AS, Denmark; Hans Christian Holm, Novozymes, Denmark; Morten Moeldrup, Novozymes, USA

Degumming of vegetable oils is divided in two main categories: alkaline refining and physical refining. Alkaline refining is known for being a robust and well proven technology. However, in alkaline refining the free fatty acids and phospholipids are eliminated by the saponification step which result in a significant oil loss. Physical refining is known from giving higher yields than alkaline refining, among other things, because of the application of phospholipases in this process which has been used for the last 20 years.

Recently, it has been suggested that it is possible to get the yield from physical refining along with the quality and robustness from alkaline refining. This is achieved by hydrolyzing the phospholipids in a separate process step inserted in the alkaline refining production line. The result is higher oil yield in the alkaline refining process coming from the di-acyl glycerols formed in the hydrolysis reaction with phospholipase C, and more neutral oil released by reduced emulsification. The yield gain is typically in the range 1–2%, depending upon the amount of phospholipids in the oil. The enzyme process can be installed between the acid pre-treatment and the saponification and it has already been confirmed in full scale operation.

In this presentation we will explore the versatility and robustness of this process and describe the details in the process with emphasis on how to set the conditions to get the optimum effect of using the enzyme step. The parameters for the process will be discussed in detail and documented with data from our experiments with different types of oil and different phospholipase enzymes.

**Insects as a Novel Source of Lecithin.** Daylan Tzompa Sosa, Ghent University, Belgium
The scope of this study was to determine whether the insect lecithin could be recovered and valorized. Lecithin was extracted from four commercially reared insects species, namely black soldier fly larvae (Hermetia illuscens), mealworm (Tenebrio molitor), house cricket (Acheta domesticus) and Jamaican field cricket (Gryllus assimilis). The interest on these species relays on their availability in the European market. Moreover, these species are the main reared species in Europe and their protein fraction is already being used in food and feed applications. Fatty acid and phospholipid profiling of the insect lecithin was performed by GC-FID and 31P-NMR to elucidate its composition. Calorimetric analysis was performed by DSC. Then, phospholipid vesicles were characterized for their size, stability (Spectrophotometry) and diffusion capacity (H-NMR). Finally, the lecithin vesicles were visualized by Cryo-SEM. The results showed that lecithin from cricket species have high levels of PC and PE. Furthermore, its fatty acid composition is enhanced by its higher PUFA content and the non-negligible presence of eicosapentaenoic acid (C20:5 - EPA). This lecithin is distinguished by the low permeability of its vesicles. This property could be illustrated by the formation of an ionic network between phospholipids, observed during differential scanning calorimetry analysis of pure lecithin. This characteristic may explain a reinforcement of the vesicle structure. In contrast, lecithin from black soldier fly larvae, showed a low concentration of phospholipids in the extract, limiting the functionality of this extract. In this presentation, the perspectives of using insects as a new source of lecithin will be presented, together with the challenges and opportunities.

**State-of-the-art Developments in Microalgal Lipids Towards Biofuel Production.** Tsuyoshi Tanaka, Tokyo University of Agriculture & Technology, Japan
Sustainable society definitely requires various types of renewable energy which suppress global CO2 release and decrease the negative effects of the released CO2 on climate change. Production of biomass-derived renewable fuels has long been studied for a century. Among biomass-derived compounds, microalgal lipids are one of the most promising resources of renewable fuels due to sophisticated photosynthetic systems of microalgae, leading to efficient biomass and lipid productions. Oleaginous microalgae accumulate the photosynthetically fixed carbon in the form of, mainly, triacylglycerols (TAGs). TAGs are readily converted to fatty acid methyl esters and hydrocarbons, useful for biodiesel and biojet fuels, with the aid of appropriate catalysts. Since capacity of TAG accumulation in the cells is dependent on the microalgal species, tremendous efforts have been devoted to discover highly oleaginous microalgae from various environment all over the world. Our screening study revealed that marine microalga Fistulifera solaris strain JPCC DA0580 and Mayamaea sp. strain JPCC CTDA0820 exhibit excellent lipid production. Both microalgae accumulate TAG with similar fatty acid compositions, mainly composed of palmitic acid, palmitoleic acid, and eicosapentaenoic acid (EPA). Up to date, we have performed multi-omics analyses, including genomics, transcriptomics, proteomics, and metabolomics studies, of these microalgae to elucidate molecular mechanism underlying their oleaginous phenotypes. In addition, we constructed a pilot-scale outdoor mass-cultivation facility for these microalgae, and are attempting to develop the robust bioprocess for year-round production of microalgal biofuels. Furthermore, we have demonstrated that metabolic engineering of these microalgae enables to produce high value-added compounds, including ω3 polyunsaturated fatty acids such as EPA, prostaglandins, and terpenoids. Besides TAGs, production of these compounds as by-products can contribute to making the microalgal biofuels economically feasible. In this presentation, I will introduce our past and current studies for efficient production and characterization of microalgal lipids useful for biofuel applications.

**Tailored Enzymatic Treatment of chlorella Vulgaris Cell Wall Leads to Effective Disruption While Preserving Lipid Oxidative Stability.** Fabiola Dionisi, Societé des Produits Nestlé - Nestlé Research, Switzerland; Greta Canelli, ETH Zurich, Switzerland; Isabelle Kuster, Migros-Industry, Switzerland; Billie Hauser, ETH Zurich, Switzerland; Patricia Murciano Martinez, Nestlé Research, Switzerland; Zhen Rohfritsch, Nestlé Research, Switzerland; Christoph Bolten, Nestlé, Germany; Lukas Neutsch, ZHAW - Zürcher Hochschule für Angewandte Wissensch, Switzerland; Alexander Mathys, ETH Zurich, Switzerland
Microalgae are primary sources of ω-3 polyunsaturated fatty acids (ω3-PUFAs), which are essential for the regulation of human biological functions and disease prevention. The microalgal cell wall can limit the bioaccessibility of protein and lipid of dried Chlorella spp. biomasses currently available on the market. Enzymatic degradation of the cell wall represents a remarkable alternative to mechanical treatments due to its mildness and specificity. This work aimed to define an optimal combination of enzymes to increase the lipid and protein bioaccessibility of C. vulgaris cells while preserving oxidative stability. Among the tested enzymes, chitinase, rhamnohydrolase, and galactanase caused the highest release of microalgae cellular material. Treatment with this enzymatic combination produced a slight increase in protein bioaccessibility, from 49.2% $\pm$ 3.9% to 58.7% $\pm$ 3.5%, but no increase in lipid bioaccessibility in comparison to the control. High-pressure homogenization (HPH) led to

61.8% ± 2.6% lipid and 59.8% ± 1.8% protein bioaccessibility. Cell integrity was preserved after enzymatic treatment, while the mean particle size was reduced from 5 to 2 μm after HPH. Oxidative stability was maintained over 3 months of accelerated shelf life in untreated and enzymatically treated C. vulgaris biomass. HPH caused drastic instability and off-flavor formation in the biomass. Tailored enzymatic treatment of C. vulgaris cell wall is a promising technique to improve the nutritional value of microalgae biomass while preserving biomass quality.

## Functional Lipids—Biocatalysis I

*Chairs In-Hwan Kim, Korea University, Republic of Korea; Tsunehiro Aki, Hiroshima University, Japan*

**Efficient Synthesis of 2-ethylhexyl Palmitate via an Immobilized Lipase-catalyzed Esterification.** In-Hwan Kim, Korea University, Republic of Korea; Suhyeon Choi, Korea University, Republic of Korea

Ethylhexyl palmitate (2-EP) from 2-ethyl hexanol and palmitic acid was synthesized successfully in a solvent free system via an immobilized lipase-catalyzed esterification. A commercial lipase (Eversa@ Transform 2.0, Novozyms) from Thermomyces lanuginosus was immobilized on Lewatit VP OC 1600, a macroporous hydrophobic carrier. Three commercial lipases, namely, Novozym 435, Lipozyme RM IM and Lipozyme TL IM, and the immobilized lipase prepared in this study were evaluated for efficient synthesis of 2-EP. Our immobilized lipase was the most effective for the synthesis of 2-EP. Optimum conditions for synthesis of 2-EP were a temperature of 55 °C and enzyme loading of 2% (of the total substrate weight). The conversion of ca. 93% was achieved under these optimum conditions. There were little differences between the conversions from two temperature protocols (55 °C for 6 h; 55 °C for 1 h followed by 45 °C for 5 h). Maximum conversion of ca. 96% was obtained when molecular sieve was added in the reaction mixture.

**Enzyme Modified Phosphatidylcholine for Use as Emulsifier to Carry Bioactive Compounds.** Hugo Garcia, Tecnologico Nacional de Mexico/IT de Veracruz, Mexico

Phospholipase A1 was employed to effect acyl exchange of soy phosphatidylcholine (PC). Medium chain, CLA and n-3 long chain fatty acids were used to replace the naturally occurring fatty acids in PC. The modified PC was employed to prepare nanoemulsions carrying curcumin or betulinic acid using high-energy methods. The nanoemulsions were studied and optimized to achieve small globule size, low PDI and high Z-potential values, all related to emulsion stability. The preparations were applied in murine models for inflammation and melanoma and the advantages of PC modification were assessed. Pharmacokinetic studies proved that the nanoemulsions prepared with modified PC were absorbed more efficiently than those made with native PC. Other applications have been approached that involved nanoemulsions to deliver other hydrophobic bioactives and insulin.

**Immobilized Lecitase Ultracatalyzed Preparation of lα-glycerylphosphorylcholine from Soy Phosphatidylcholine.** Byung Hee Kim, Sookmyung Women's University, Republic of Korea; Yejin Song, Sookmung Women's University, Republic of Korea; Seoye Roh, Sookmyung Women's University, Republic of Korea; Jihyun Hwang, Sookmyung Women's University, Republic of Korea; min-Yu Chung, Korea Food Research Institute, Republic of Korea; In-Hwan Kim, Korea University, Republic of Korea

The aim of this study was to prepare a cognitive enhancer L-α-glycerylphosphorylcholine (L-α-GPC) using an immobilized Lecitase Ultra (phospholipase A1) to catalyze the hydrolysis of soy phosphatidylcholine. Immobilization of Lecitase Ultra on Lewatit VP OC 1600 (macroporous polymethacrylate cross-linked with divinylbenzene) provided the highest fixation level (83.1 g/100 g) and greatest catalytic activity achieving 100 g/100 g L-α-GPC within 20 h and was therefore selected as the optimal system for biocatalysis. Immobilization of Lecitase Ultra increased its positional specificity compared to free Lecitase Ultra, as shown by a decrease in the production of the phosphocholine byproduct. Under the optimal conditions determined by response surface methodology, phosphatidylcholine was completely hydrolyzed to L-α-GPC and required a simple purification via phase separation of the biphasic media to obtain a yield of ∼26.4 g L-α-GPC from 100 g phosphatidylcholine, with a purity of 98.5 g/100 g. Our findings suggest a possibility of using the immobilized Lecitase Ultra as a new biocatalyst for the L-α-GPC production.

**Improvement of Lipid Productivity of aurantiochytrium Sp. by Genome Editing.** Kenshi Watanabe, Hiroshima University, Japan; Charose Marie Ting Perez, Hiroshima University, Japan; Tomoki Kitahori, Hiroshima University, Japan; Kosuke Hata, Hiroshima University, Japan; Masato Aoi, Hiroshima University, Japan; Hirokazu Takahashi, Hiroshima University, Japan; Tetsushi Sakuma, Hiroshima University, Japan; Yoshiko Okamura, Hiroshima University, Japan; Yutaka Nakashimada, Hiroshima University, Japan; Takashi Yamamoto, Hiroshima University, Japan; Keisuke Matsuyama Nagase & Co., Ltd., Japan; Shinzo Mayuzumi, Idemitsu Kosan Co., Ltd., Japan; Tsunehiro Aki, Hiroshima University, Japan

Strains of genus Aurantiochytrium accumulate significant amounts of polyunsaturated fatty acids and carotenoids and are ideal as industrial producers. Various efforts such as chemical mutagenesis and homologous gene recombination have been carried out to improve lipid productivity, but the specificity and efficiency of such conventional methods are low, which hinders the progress of research. Here, the CRISPR-Cas9 system, which is a genome editing technology, was applied as an alternative molecular breeding technology for Aurantiochytrium species to accelerate metabolic engineering. Combined with the CRISPR-Cas9 system, the efficiency of homologous recombination for specific gene knock-ins has increased by more than 10-fold. As a result of disrupting genes associated with β-oxidation of fatty acids by this improved method, genome-edited strains with higher fatty acid productivity were isolated. This is the first time to demonstrate that the CRISPR-Cas9 system is effective in molecular breeding of Aurantiochytrium strains and improves lipid productivity.

**Optimization and Analysis of an Enzymatic Glycerolysis of Soybean Oil to Produce Diacylglycerol-enriched Oil in a Supercritical Carbon Dioxide System.** Nazanin Vafaei, University of Manitoba, Canada; Martin G. Scanlon, University of Manitoba, Canada; Curtis. B Rempel, Canola Council of Canada, Canada; N A Michael Eskin, University of Manitoba, Canada; Peter Jones, University of Manitoba, Canada

This research addresses an alternative green methodology for the synthesis of diacylglycerol-enriched (DAG) oil by enzymatic glycerolysis of soybean oil in a supercritical carbon dioxide (SCCO2) medium. DAG oil has been recently highlighted as a healthy oil. Phosphorous nuclear magnetic resonance (31P-NMR), an eco-friendly, fast, and precise analytical technique, was used for analysis of the different isomers of partially esterified glycerols (PEGs), free fatty acids (FFAs), and tocopherols of the soybean oil-based DAG oil (SDO) mixture taken from the reaction. After 8 hours (h) reaction, enzymatic glycerolysis in SCCO2 increased dramatically (1700%) the percentage change of total diglyceride (DG) in SDO compared to the same reaction at atmospheric pressure. A total DG content of 44% (w/w), containing 60% of the healthier 1,3-DG isomer versus 40% 1,2-DG, was obtained at a pressure of 80 bar, temperature of 60 °C, and an enzyme load of 10% (w/w) following 8 h of enzymatic glycerolysis. A mathematical model was developed to find the relationship between the total DG production and the independent variables (i.e., pressure, time, enzyme load and temperature). To validate the efficacy of the model, the Least Square technique was employed and the value of $R2$ was 0.94 which shows that the model is well fitted to the experimental results.

**Preparation and Bio-function of Polyunsaturated Phosphatidylglycerol.** Masashi Hosokawa, Hokkaido University, Japan; Liping Chen, Hokkaido University, USA

Phosphatidylglycerol (PG) is a functional phospholipid (PL), which has many physiological functions and shows excellent liposome forming activity. However, naturally occurring PG, especially n-3 polyunsaturated fatty acids enriched PG (n-3 PUFA-PG), is low in content, resulting in a scarcity of industrial bio-resources. We therefore established a n-3 PUFA-PG preparation method using marine phospholipids such as salmon roe lipids via phospholipase D (PLD)-mediated transphosphatidylation. The yield of n-3 PUFA-PG from salmon roe total lipid in the aqueous system without organic solvent reached 96.4 mol%, following a 24 h reaction. Synthesized n-3 PUFA-PG significantly reduced the total and non-HDL cholesterols in the serum of diabetic/obese KK-Ay mice. In the mice fed n-3 PUFA-PG, but not n-3 PUFA-TAG, hepatic lipid content was markedly alleviated depending on the neutral lipid reduction. Furthermore, n-3 PUFA-PG showed more potent anti-inflammatory activity than n-3 PUFA-phosphatidylcholine (PC) in lipopolysaccharide-stimulated macrophage-like RAW264.7 cells through Nrf-2 activation. Notably, n-3 PUFA-PG-treated cells showed increased n-3 PUFAs compared to n-3 PUFA-PC treatment. These results indicate that n-3 PUFA-PG prepared from marine PL by PLD is the highly functional lipid.

## General Biotechnology

*Chair Todd Underiner, P&G, USA*

**A Journey of Lipid Biotechnology from Single Cell Oil to Lipidomics.** Suk Hoo Yoon, Samyang Cooperation, Republic of Korea

Fats and oils have traditionally been obtained from animals and plants by conventional mechanical and/or chemical processes. With advancement of biological technology, lipids could also be obtained from microorganisms, so called single cell oils, practically. Fats and oils could be extracted, refined, and processed partially by biotechnological means. The novel lipids such as structured lipids can be produced by enzymatic and microbial technology. The developments of new sources of fats and oils for food purposes, and effective processing techniques have been pursued to increase the efficiency of utilization. The total quality and oxidative stability of fats and oils are influenced by the minor components present in fats and oils, and the kind and amount of minor components are substantially dependent upon the processing conditions of oleaginous materials. Lipids have long been considered to relate with obesity in living organisms, and several metabolites produced from lipid anabolism and catabolism are

characterized in the obese and non-obese animal subjects.

**Development of a New Innovative Process for the Production of Bioactive Peptides Resulting from the Enzymatic Hydrolysis of Bovine Hemoglobin: Electrodialysis with Bipolar Membranes.** Mira ABOU DIAB, Laval University, Canada; Laurent Bazinet, Laval University, Canada; Naima Nedjar, Institut Charles Violette, France

Bovine cruor, a slaughterhouse waste, is mainly composed of hemoglobin, a protein rich in bioactive peptides after its hydrolysis. However, during conventional hydrolysis, chemical agents are necessary to adjust and regulate the pH of the protein solution and the mineral salt content of the final hydrolysate was consequently high. The aim of this project was to prove the feasibility of enzymatic hydrolysis of bovine hemoglobin by electrodialysis with bipolar membrane (EDBM), to obtain bioactive peptides without the use of chemicals and with a low salt content. Bipolar/monopolar membranes (anionic (AEM) and cationic (MCP)) configurations using the $H+$ and OH- generated by the bipolar membranes to regulate the pH were investigated. EDBM-MCP allowed the production of hydrolysates containing a low concentration of mineral salts but with fouling formation on MCP, while EDBM-AEM allowed the production of hydrolysates without fouling but with a similar salt concentration than the conventional hydrolysis (control). Hydrolysis in EDBM showed the same enzymatic mechanism, ''Zipper'', identical to that obtained in control. By the «Zipper» mechanism, EDBM, whatever the configuration of MCP or AEM, allowed for a recovery rate of the alpha and beta chain of bovine hemoglobin greater than 91%. Furthermore, following the identification of peptide sequences by mass spectrometry, EDBM produced 17 bioactive peptides, including neokyotorphin ($\alpha$137–141), showing several biological activities of great interests for the food industry in the framework of a circular economy. The hydrolysates derived from hydrolysis by EDBM showed excellent antibacterial activity against six bacterial strains, antioxidant activity and for the first-time their antifungal activity was demonstrated, and that against five strains of molds and yeasts. In this study, electrodialysis with bipolar membrane (EDBM) was demonstrated for the first time as an eco-efficient and innovative technology to produce peptide hydrolysates from enzymatic hydrolysis of bovine hemoglobin with low mineral salt concentration.

**ISO TC 34/SC 16 Horizontal Methods for Molecular Biomarker Analysis—International Standards for Molecular Biomarker Analysis/isothermal Nucleic Acid Amplification Methods.** Michael Sussman, USDA, Agricultural Marketing Service, USA

Harmonized, easy to handle methods of analysis with defined patterns and known nomenclatures bring more customers to the market. The International Organization for Standardization (ISO.org) was formed in 1946. It is an independent, non-governmental voluntary consensus standard body based in Geneva, Switzerland with a membership of 165 national standards bodies. The US ISO member is the American National Standards Institute (ANSI.org) a consortium of US standardization organizations. There are 44 participating countries. The US delegation responsible for developing the US position for standards development in agricultural molecular biomarker analysis was delegated to the American Oil Chemist's Society (AOCS.org) by ANSI. The AOCS US TAG also hosts the TC 34/SC 16 international secretariat. TC 34/SC 16 has published 29 standards with another 10 under development. SC 16 is working on a standard providing general criteria for development and validation of isothermal nucleic acid amplification analytical methods. Isothermal nucleic acid amplification-based methods are now being used to amplify DNA for detection, identification, quantification and analysis of biomarkers in food and food products. Isothermal nucleic acid amplification is a constant temperature polymerase mediated chain reaction. Some advantages are no need for precision thermocycling instruments, single assay printable paper chromatographic format permits for ease of use, in some cases use of unpurified cell extract as substrate and only a few preparation steps. The new ISO standard is applicable to food, feed, plant matrices, and their propagules, plant pathogens and animals and provides guidance for specific isothermal amplification technologies.

**The Effect of Protein-surfactant Interactions on the Stabilization of Foam in the Presence of Oil.** Jason Berberich, Miami University, USA Kayla; Thompson, Miami University, USA; Jared Coffin, Miami University, USA; Elizabeth Caraballo-Torrealba, Miami University, USA; Juan Velasquez, Procter & Gamble, USA

A better understanding of the interactions between proteins and surfactant mixtures is needed to improve product development and formulation in the chemical, pharmaceutical, food and cosmetic industries. Mixtures of proteins and surfactants can help to increase the stability of foams and emulsions by improving adsorption properties and surface elasticity. Depending on the ratio of protein to surfactant in solution, proteins and surfactants interact to form complexes that may have different structures and adsorption properties. The chemical properties of the protein and surfactant can affect the structure of the protein-surfactant complex which can significantly impact their surface properties. We investigated foam production and stability, in the presence of oil, of protein-surfactant mixtures with varying ratios of surfactant to protein. Circular dichroism and tryptophan fluorescence were used to characterize the protein structure in the protein-surfactant complexes. Pyrene fluorescence was used to characterize

the formation of protein-surfactant aggregates and pendant drop tensiometry was used to measure the surface pressures of the complexes. The location of the protein in the complex air/water/oil mixtures was also determined using confocal laser scanning microscopy. Results show the stabilization of foam production over a range of conditions upon the addition of b-lactoglobulin or bovine serum albumin. No protein was observed to bind to the oil interface at high ratios of surfactant to protein. Presence of protein was found to reduce the surface tension of the surfactant mixtures at lower surfactant concentrations. Possible mechanisms for the stabilization of foam production in the presence of oil will be discussed.

## Oleochemicals- Biocatalysis II

*Chairs Lu-Kwang Ju, The University of Akron, USA; Jun Ogawa, Kyoto University, Japan*

**Enzymatic Interesterification of Palm-based Oil Catalyzed by sn-1,3 Specific Lipase: Acyl Migration and Physical Properties.** Yong Wang, Jinan University, China (People's Republic); Wan Jun Lee, Jinan University, Malaysia; Zhen Zhang, Jinan University, China (People's Republic)

Acyl migration of fatty acid at sn-2 is often observed alongside enzymatic interesterification (EIE), causing the loss of lipase selectivity towards the acyl group at sn-1,3. In this study, oil blend consisting of palm stearin (PST) and palm olein (POL) was interesterified via EIE using a packed bed reactor. Characterization in terms of the triacylglycerol (TAG) compositions, sn-2 fatty acid distributions, and solid fat content profiles were performed. Under similar reaction conditions, different interesterification degree (ID) were obtained according to the various blend ratios. Using the same mass ratio of substrates (POL:PST of 9:1), EIE reaction time and temperature affected the ID and the change in the fatty acyl group at sn-2 position. Under the reaction time of 46 min, an ID of 94.41% was acquired while at 80°C, degree of acyl migration at sn-2 was 92.87%. EIE with high acyl migration exhibited lower crystallization rate than that of EIE with low acyl migration. However, the effect of acyl migration on crystal polymorphism and oxidative stability was insignificant. Outcomes from this study are meaningful for the establishment of a theoretical basis for a controlled positional specific EIE that is catalyzed by sn-1,3 specific lipase.

**Molecular Breeding of the ω3-docosapentaenoic Acid-producing Microorganism aurantiochytrium Sp. T7.** Jun Ogawa, Kyoto University, Japan; Brian K. H. Mo, Kyoto University, Japan; Tomoyo Okuda, Kyoto University, Japan; Ayami Hatano, Kyoto University, Japan; Keisuke Matsuyama, Nagase & Co., Ltd., Japan; Akinori Ando, Kyoto University, Japan

Omega-3 polyunsaturated fatty acids (ω3-PUFAs) are reported to have various physiological functions. While the physiological roles of EPA, DHA, and ALA, have been well studied because of their sufficient natural supply, the biological roles of rare ω3-PUFAs such as SDA (18:4ω3), DPA (22:5ω3), and ETA (20:4ω3) are unclear due to a lack of known sources. For example, while studies have shown ω3-DPA to be a component of natural oils extracted from earless seal, its content was less than 5% of total lipids. Thus, recent research has focused on ω3-DPA production by alternative sources such as oleaginous bacteria, fungi, plants, and microalgae.

We previously reported the isolation of a microorganism Aurantiochytrium sp. strain T7 that accumulates a significant amount of ω3-DPA, and optimized its culture conditions.

In this study, we constructed a transformation system to elucidate the mechanism of ω3-DPA biosynthesis in the T7 strain. We constructed an expression vector containing a zeocin resistance gene as a selection marker and a β-glucuronidase (GUS) gene as an expression reporter gene. We transformed this vector into the T7 strain by the biolistics method, and confirmed stable GUS activity in the obtained transformants. We applied this transformation system to modify the fatty acid profile and to analyze the ω3-DPA biosynthetic pathway of Aurantiochytrium sp. strain T7.

**Tailoring Oil Palm Planting Materials for the Oleochemical Industry.** Rajinder Singh, Malaysian Palm Oil Board, Malaysia; Leslie Low Eng-Ti, Malaysian Palm Oil Board, Malaysia; Meilina Ong Abdullah, Malaysian Palm Oil Board, USA; Ravigadevi Sambanthamurthi, Malaysian Palm Oil Board, Malaysia; Mohd Din Amiruddin, Malaysian Palm Oil Board, Malaysia; Yeong Shoot Kian, Malaysian Palm Oil Board, Malaysia; Mohamad Arif Abd Manaf, Malaysian Palm Oil Board, Malaysia; Ghulam Kadir Ahmad Parveez, Malaysian Palm Oil Board, Malaysia

Palm oil and palm kernel oil are raw materials from which basic oleochemicals, namely fatty acid and fatty acid methyl esters are derived. The fats and oils utilized in the oleochemical industry are those composed of fatty acids with carbon chain lengths of C8-C18, especially C12-14 of which coconut oil and palm kernel oil are the principal representatives and C16-C18 which includes tallow and palm oil. As such, to cater to the oleochemical industry, oil palm breeding has also been custom-tailored to develop the appropriate planting materials which can yield higher levels of selected fatty acids. Breeding lines with kernel content up to 10%, compared to 5% observed in current commercial material, which will yield higher lauric acid are being developed via conventional breeding. High concentrate oleic

acid is an important chemical requirement of the oleochemical industry. Palms with higher oleic acid content have also been identified. However, DNA markers are required to fix the traits in advanced breeding lines, in order to develop the new and improved varieties faster for this perennial crop. In this respect progress has also been made via MPOB's genome research in identifying genomic loci linked to kernel content and the different fatty acids in selected breeding populations. The presentation will also describe how these tools are being used to aid in the development of planting materials with higher content of selected fatty acids to meet the long-term requirements of the oleochemical industry for the key raw materials.

## Plant and Algae Lipid Biotechnology and Genomics

*Chairs Jay Shockey, SRRC, ARS, USDA, USA; Timothy Durrett, Kansas State University, USA; Jinyue Sun, Institute of Agro-Food Science and Technology/Key Laboratory of Agro-Products Processing Technology of Shandong Province, Shandong Academy of Agricultural Sciences, Jinan 250100, China, China (People's Republic)*

**2-acetyl-1,3-diacyl–sn-glycerols, Novel Plant Lipids with Diverse Potential.** Mark Smith, Agriculture and Agri-Food Canada, Canada

Acetyl-triacylglycerols resemble conventional TAG but one fatty acid is substituted by acetic acid. Two types of acetyl-TAG have been reported from seeds, 3-acetyl-1,-2-diacyl-sn-glycerols (sn-3-acetyl-TAGs) from plants in the genus Euonymus (Celastreae), and 2-acetyl-1,-3-diacyl-sn-glycerols (sn-2-acetyl-TAGs) from species in the milkwort family (Polygalaceae). The sn-3-acetyl-TAGs are of considerable interest as potential biolubricants and fuels due to their reduced viscosity and superior low temperature fluidity. Whereas the pathway of sn-3-acetyl TAG biosynthesis in Euonymus species has been characterized and the enzyme responsible for the acetylation of the sn-3 position of DAG has been identified, the pathway for sn-2 acetyl-TAG synthesis remains to be discovered.

Due to the location of the acetate group, sn-2-acetyl-TAGs may have additional potential in nutraceutical or pharmaceutical applications. In addition to the presence of acetate, natural sources of these oils are also diverse in fatty acid composition. We have conducted further characterization of oils rich in sn-2 acetyl TAG and an oil reported to accumulate moderate levels of both sn-2 and sn-3-acetyl-TAGs using techniques such as FTIR, MALDI-TOF mass spectrometry and NMR. Data confirm the presence of conjugated hydroxydienoic fatty acids including coriolic acid (13-HODE) and of sn-2 acetyl TAG in the oil from seeds of the violet tree (Securidaca longipedunculata). Purification and preliminary feeding studies conducted with sn-2 acetyl-TAG from Polygala tenuifolia suggests potential novel applications for these unusual oils.

**Biochemical Characterization of a Wax Synthase from a Green Microalga chromochloris Zofingiensis.** Guanqun (Gavin) Chen, University of Alberta, Canada; Yang Xu, University of Alberta, Canada; Xue Pan, University of California, Riverside, USA; Junhao Lu, University of Alberta, Canada; Juli Wang, University of Alberta, Canada; Qiyuan Shan, University of Alberta, Canada; Jake Stout, University of Manitoba, Canada

Wax synthase (WS) catalyzes the last step in wax ester biosynthesis in green plants. Two unrelated subfamilies of WS including the bifunctional acyltransferase and plant-like WS have been reported, but the latter is largely uncharacterized in microalgae. Here, we functionally characterized a putative plant-like WS from the emerging model green microalgae Chromochloris zofingiensis (CzWS1). Our results showed that plant-like WS evolved under different selection constraints in plants and microalgae, with positive selection likely contributing to functional divergence. Unlike jojoba with high amounts of wax ester in seeds and a highly active WS enzyme, C. zofingiensis has no detectable wax ester but a high abundance of WS transcripts. Co-expression analysis showed that C. zofingiensis WS has different expression correlations with lipid biosynthetic genes from jojoba and may have divergent functions. In vitro characterization indicated CzWS1 had diacylglycerol acyltransferase (DGAT) activity along with WS activity, and overexpression of CzWS in yeast and Chlamydomonas reinhardtii could affect triacylglycerol accumulation in yeast and microalgae. Moreover, biochemical and bioinformatic analyses also revealed the crucial role of the C-terminal region of CzWS1 in enzyme function. Taken together, the CzWS1 encodes a functional plant-like WS with both WS and DGAT activities, which may contribute to storage lipid biosynthesis and represents a potential target for engineering wax production in microorganisms.

**Generation of Camelina Mid-oleic Acid Seed Oil by Identification and Stacking of Fatty Acid Biosynthetic Mutants.** Nicholas Neumann, Kansas State University, USA; Tara Nazarenus, University of Nebraska-Lincoln, USA; Jose Aznar-Moreno, Kansas State University, USA; Luca Comai, UC Davis, USA; Edgar Cahoon, University of Nebraska-Lincoln, USA; Timothy Durrett, Kansas State University, USA

The fatty acid composition of seed oil often needs to be improved to enable use for specific applications such as food processing, biofuels, or biolubricants. We used high-throughput gas chromatography to screen a mutant population of camelina (Camelina sativa), for lines with altered seed oil fatty acid composition. By leveraging knowledge of fatty acid synthesis in

Arabidopsis thaliana, we identified mutations in homeologs of FATTY ACID ELONGASE1 (FAE1), FATTY ACID DESATURASE2 (FAD2), FATTY ACID DESATURASE3 (FAD3), and β-KETO-ACYL-ACP SYNTHASE II (KASII; FAB1). The mutations affected highly conserved amino acid residues in the encoded proteins. The ability of the mutations in FAE1, FAD2 and FAD3 to alter enzyme function was demonstrated by comparing in vivo activities of wild-type and mutant alleles in yeast. As camelina has a hexaploid genome, the effect of a mutation in one of the three homeologs for each gene resulted in no or less severe growth phenotypes. We obtained mid-oleic oils with nearly 40% oleic acid and reduced very long-chain (≤C20) fatty acid content by crossing mutant lines to obtain a fae1c/fad2a/fae1a/fad3a quadruple mutant. The resulting mid-oleic acid oil had improved oxidative stability due to reductions in polyunsaturated fatty acid content, increasing its utility for biofuels and other applications.

**Impairment of Polyphosphate Biosynthesis Triggers Triacylglycerol Biosynthesis via Either the PDAT or DGTT1 Catalyzed Pathway in the Model Alga Chlamydomonas Reinhardtii.** Yantao Li, the Institute of Marine and Environmental Technology (IMET), the University of Maryland, USA; Yi-Ying Lee, The Institute of Marine and Environmental Technology (IMET), the University of Maryland, USA

Polyphosphate has been found in all kingdoms of life as phosphorus storage. Compared to the well-studied bacteria polyphosphate metabolism, our understanding of microalgal polyphosphate metabolism is lagging behind. In this study, polyphosphate biosynthesis and its interaction with lipid biosynthesis was explored in the model alga Chlamydomonas reinhardtii using a mutant defective in the vacuolar transporter chaperone 1 gene (VTC1). Deletion of the VTC1 gene (Δvtc1) downregulated the expression of the phosphate transporter genes and impaired phosphate uptake. As a result, the Δvtc1 mutant accumulated little polyphosphate. By contrast, the Δvtc1 mutant accumulated more TAG under phosphorus depleted conditions, resulting in a 50% increase in TAG content. This was accompanied by overexpression of the phospholipid: diacylglycerol acyltransferase (PDAT) but not diacylglycerol acyltransferase (DGAT) genes. Interestingly, knockout of PDAT in the Δvtc1 background (Δvtc1ΔPDAT) increased TAG content drastically by 182.3%, suggesting compensation of TAG biosynthesis by non-PDAT mediated pathways. Further analysis of the Δvtc1ΔPDAT mutant revealed one of the DGAT genes, DGTT1 was responsible for enhanced TAG biosynthesis. These data suggest that impairment of polyphosphate biosynthesis induces TAG biosynthesis via PDAT, while further knockout of PDAT triggers compensation by DGTT1. Impairment of polyphosphate biosynthesis also affected the fatty acid composition of TAG, promoting accumulation of mono- and polyunsaturated fatty acids at the expense of saturated fatty acids. Manipulations of DGATs for polyunsaturated fatty acids production and their biotechnological applications were also discussed.

**Pennycress to Covercress: Rapid Crop Domestication with Gene Editing Technologies.** Michaela McGinn, Covercress, USA; Rahul Pathakar, Covercress Inc, USA

Pennycress (Thlaspi arvense) is a common Brassica found throughout temperate regions of the world with a long list of unique attributes including an overwintering growth habit, relatively short life cycle, and naturally high oil and protein content in the up to 40 grams of seed produced per plant. CoverCress Inc. is utilizing cutting-edge gene editing technologies to rapidly domesticate and tailor this plant to serve as a cash cover crop. Elite pennycress varieties are subjected to multiple targeted genetic alterations resulting in a new variety of pennycress, Covercress, which possesses a novel lipid profile, higher seed meal quality, and earlier maturity time with no detectable mutagenesis reagent in two generations. Covercress can be planted throughout the Midwest Cornbelt within the existing corn/soybean rotation, being planted over standing corn and harvested before soybeans are planted. Using the wealth of knowledge from closely-related model plant Arabidopsis thaliana and what has been gleaned from standard mutagenesis and breeding experiments, new gene editing technologies can be used to quickly and effectively domesticate and modify high yielding pennycress germplasm into domesticated and tailor-made Covercress varieties ready to field test in under a year.

**Synthetic Biology Application for Development of High-value Oil Traits.** Edgar Cahoon, University of Nebraska-Lincoln, USA; Kiyioul Park, University of Nebraska, USA; Hyojin Kim, University of Nebraska, USA; Tom Clemente, University of Nebraska, USA

Synthetic biology offers tools for introduction of increased genetic diversity into crop plants with unprecedented speed and accuracy. We are applying these tools to develop crops, including soybean, camelina, maize, and sorghum, with novel compositional and agronomic traits to improve their economic value and productivity. We use a design-build-test-learn (DBTL) cycle to advance potential trait genes through a development pipeline from lab-to-greenhouse-to-field. Engineered crops arising from this pipeline are evaluated for compositional properties and alterations in metabolomes to iteratively improve synthetic biology designs. One of our targets is the development of soybeans for aquaculture feed that produce seed oils enriched in the high-value carotenoid astaxanthin, the vitamin E-type antioxidants tocotrienols, and the omega-3 polyunsaturated fatty acids eicosapentaenoic

acid (EPA) and docosahexaenoic acid (DHA). We use modular, multi-gene assembly systems to develop expression constructs that produce all of these trait targets in seeds of single engineered plants. Our initial prototype developed by introduction of seven trait genes under seed-specific promoters into soybean succeeded in yielding astaxanthin-, tocotrienol-, and EPA-enriched seeds. We are also working to optimize astaxanthin production in seeds. To speed the DBTL cycle, we are using Nicotiana benthamiana as a platform for rapid transient evaluation of astaxanthin biosynthetic genes. We then use camelina, based on its simple transformation protocol and short lifespan, as an oilseed biotechnology platform for testing candidate genes under greenhouse and field conditions and to obtain oil for functional analyses. Other ongoing synthetic biology research projects include the development of sorghum with vegetative oils as a biofuel coproduct and maize with altered root exudates to promote interactions with beneficial soil microbes.

## Gene Editing Technology and Advancements in Agriculture

*Chair Tim Ulmasov, CoverCress, Inc., USA*

**Covercress—a Novel Oilseed Winter Crop with Canola-like Composition That Helps Sequester carbon and Prevent Soil Erosion.** Tim Ulmasov, CoverCress, Inc., USA

There is an urgent need for reducing carbon footprint and other detrimental impacts of civilization on the environment. One of the solutions proposed in agriculture are cover crops that are generally grown between regular cropping seasons and provide significant benefits such as enhanced soil health and increased carbon sequestration. The main problem with lack of widespread cover crops adoption is in their economics, as most farmers avoid planting them due to guaranteed costs and uncertain returns from the benefits to the following crop. This results in misplaced economic incentive where the society greatly benefits from increased cover crop use, but most farmers are not prepared to pay for that. To address this dilemma, CoverCress, Inc. developed a novel oilseed crop that can be used as a feedstock for bioenergy production, as well as for human and animal consumption. The main advantage is that it doesn't compete for land with any of the established crops, resulting in low Carbon Intensity (CI=~30) score of the oil and meal. Based on known weed field pennycress (Thlaspi arvense), it can be used to produce oil with attractive properties for renewable diesel, jet fuel or food use, as well as protein-rich seed meal with that can be used for animal feed or as a good source of plant-based protein. Pennycress seeds have high oil content (~32%) with the lowest saturated fat content among commercially available plant-based oils (< 4%). The winter annual life cycle of pennycress enables planting in early fall immediately following corn harvest and collecting the grain prior to soybean planting in mid-to-late May. Using a combination of conventional breeding and CRISPR-mediated genome editing we were able to rapidly domesticate wild pennycress into CoverCress, a low CI canola-like crop that can be launched in central Midwest as soon as in fall of 2021.

**Delivering Plant-based Innovations for a Better World.** Travis Frey, Calyxt

The future demands healthy and sustainable plant-based innovation, which is where Calyxt comes in to play a role. We are revolutionizing the way the world uses plants to solve problems with cutting-edge proprietary breeding technologies, including TALEN® gene editing technology. We are making possible what farmers and botanists have been doing for hundreds of years by choosing the best plants and breeding them to make stronger, more uniform and productive crops such as oat, hemp, and soybean. We accelerate this process by enhancing the unique characteristics that naturally exist in each plant to develop breakthrough products that deliver benefits like climate resistance, consumer health, inputs for advanced materials and much more. At Calyxt, we're exploring the possibilities of unleashing the potential of plants of which the opportunities are endless.

**Gene Editing Approaches to Improve Seed Composition of Oilseed Crops.** Anthony Kinney, Corteva Agriscience, USA

Output traits research creates value for growers, processors, food companies and consumers by improving the quantity or quality of seed components such as protein and oil. Corteva's oilseed output trait research aims to create highly differentiated soybeans, canola and other oilseeds—a goal aligned with our strategic intent of innovating to feed the world. More than 75 percent of the protein in our food originates from soy-based feed used for meat, milk and egg production. Demand for soy is rising along with global population and in response to a growing developing world market for animal protein. At the same time, rapid growth in consumer interest in plant-based foods could fuel disruptive demand for high-quality plant proteins derived from soy and other protein crops. Soybeans also provide about 30 percent of the world's vegetable oil, making them the second most important source of food-grade oil; this use is a significant determinant of seed value. Corteva's efforts to increase oilseed protein and oil content, and to improve amino acid and fatty acid composition, leverage our expertise in CRISPR-cas gene editing, crop plant transformation, and plant metabolic engineering.

**How CRISPR Technology Will Help You Eat More Fruits and Vegetables.** Aaron Hummel, Pairwise, USA
Consumption of fruits and vegetables—the most effective solution to malnutrition—can be limited by low production or high cost in some countries and by poor quality characteristics or inconvenient preparation in others. Americans, for example, consume only half the recommended daily intake of fruits and vegetables despite widespread availability and high calorie diets. Pairwise is using genome editing to address these challenges regionally and globally by making healthy food more convenient, affordable, and sustainable. To fulfill this mission, we have assembled a world class portfolio of CRISPR editing technologies and a state-of-the-art R&D capability focused on delivering differentiated products to the fresh produce aisle. With these tools we have made new fresh produce products in leafy greens and berries to increase the variety of nutritious fresh foods available for the consumer. This presentation will describe the Pairwise vision to deliver new products in fresh produce, the development process for our products, and the technology platform that is making it possible.

**Summary of the SECURE Rule, a Revision of the USDA Biotechnology Regulation.** Neil Hoffman, Animal Plant Health Inspection Service/Biotechnology Regulatory Services, USA
In keeping with the directive in Executive Order 13874 (Modernizing the Regulatory Framework for Agricultural Biotechnology Products) to adopt regulatory approaches that are proportionate to risk and avoid arbitrary distinctions across like products, the United States Department of Agriculture (USDA) revised its biotechnology regulations by promulgating the Sustainable, Ecological, Consistent, Uniform, Responsible, and Efficient (SECURE) rule. Specifically, the SECURE rule: 1) establishes exemptions for plants modified by genetic engineering where the modification could otherwise have been made through conventional breeding; 2) uses risk posed by the introduced trait to determine whether an organism is regulated, rather than relying on whether the organism was developed using a plant pest; and 3) provides a mechanism for a rapid initial review to efficiently distinguish plants developed using genetic engineering that do not pose plausible pathways to increased plant pest risk from those that do. As a result of the focused oversight on potentially riskier crops developed using genetic engineering, USDA is expected to improve the efficiency and effectiveness of its oversight program. The reduced regulatory burden is expected to promote innovation by expanding the number and diversity of developers to include smaller businesses and academics and to increase the number and variety of traits being developed through biotechnology.

## Biorenewable Polymers

*Chairs Richard Ashby, USDA/ARS/ERRC, USA; Eric Cochran, Iowa State University, USA*

**From Cashew Nutshell Liquid to Aromatic Bio-based Latex.** Sylvain Caillol, ICGM, France
Cardanol, which is a natural phenolic oil, is issued from Cashew Nutshell Liquid (CNSL), a non-edible renewable resource, co-produced from cashew industry in large commercial volumes (1Mt p.a.). Cardanol is non-toxic and particularly suitable for the addition of aromatic renewable resources in polymers and materials. We recently reported various routes for the synthesis of di- and poly-functional building blocks derived from cardanol thereafter used in polymer syntheses [1]. We especially synthesized a new radically polymerizable cardanol-derived monomer. Hence, we synthesized cardanol-based aromatic latex by radical aqueous emulsion (and miniemulsion) polymerization. We also synthesized UV-reactive cardanol-derived latex for styrene-free coating applications. The latexes were colloidally stable with monomodal particle size distributions and mean particle diameters ranging from 100 to 250 nm. The physical and chemical properties of the resulting polymer films were studied by thermogravimetric analyses and rheology [2,3]. Eugenol (4-allyl-2-methoxyphenol), a major component of clove oil, is an aromatic renewable resource with potential to replace some petroleum-based aromatic monomers. We interestingly synthesized a new platform of eugenol-derived methacrylates and studied for the first time their reactivity in radical aqueous emulsion (and miniemulsion) polymerization. The resulting latexes were stable and featured an average particle diameter of 40–50 nm. These results open the door to the formulation of new bio-based aromatic latexes with potential applications in adhesives and coatings [4].

References
[1] C Voirin, S Caillol, N V. Sadavarte, B, V. Tawade, B Boutevin, PP Wadgaonkar, Polymer Chemistry, 5 (2014), 3142–3162
[2] V Ladmiral, R Jeannin, K Fernandes Lizarazu, J Lai-Kee-Him, P Bron, P Lacroix-Desmazes, S Caillol, European Polymer Journal, 93 (2017), 785–794
[3] WS Jennifer Li, C Negrell, V Ladmiral, J Laikeehim, P Bron, P Lacroix-Desmazes, C Joly Duhamel, S Caillol, Polymer Chemistry, 9 (2018), 2468–2477

**Sustainable Synthesis of Bio-based Recyclable Thermosets.** Audrey Llevot, Organic Polymer Chemistry Laboratory (LCPO), Bordeaux, France; Pia Löser, Karlsruhe Institute of technology, Germany; Michael Meier, Karlsruhe Institute of technology, Germany

The awareness of environmental deterioration and our dependency on depleting fossil feedstocks force research to find innovative solutions in order to design a more sustainable future. Therefore, this presentation focusses on the sustainable synthesis of bio-based monomers for recyclable thermosetting polymers.

Vanillyl alcohol was used as renewable substrate and modified by an allylation and, in some cases, epoxidation procedure. In order to introduce recyclability into the final polymer networks, the modified vanillin building blocks were subsequently coupled to form dimeric structures bearing cleavable linker groups. The dienes were later polymerized by thiol-ene reaction and the diglycidyl ethers were investigated as substitutes of bisphenol A for the preparation of epoxy resins, in both cases by combination with terpene-based monomers. Limonene was used for the synthesis of bio-based diamines exhibiting a similar structure as isophorone diamine. A terpene-based trithiol was also synthesized from myrcene.

The thiol-ene polymerization of the vanillin-based cleavable diene monomers with the myrcene-based trithiol resulted in transparent cross-linked films. Epoxy resins were prepared from the vanillin-based diglycidyl ether monomers and the terpene-based diamines. The terpene-based diamines proved to be suitable hardeners for epoxy resin synthesis. In both cases, the thermomechanical properties of the obtained polymer networks were studied. This study enabled the determination of the compatibility of the linker nature with the employed chemistry.

Finally, different degradation conditions were explored to establish a protocol for the controlled degradation of the cross-linked polymers. In a first step, the cleavable monomers were used as model systems to identify suitable degradation conditions. Subsequently, the most promising conditions were transferred to the polymer films to prove the degradability of the polymer network.

**Synthesis and Characterization of Betulin-based Thermoplastics and Thermosets.** Alexandra Chong, Rowan University, USA; Silvio Curia, Rowan University, USA; Melissa Gordon, Lafayette College, USA; Lindsay Soh, Lafayette College, USA; Joseph Stanzione, Rowan University, USA

Betulin, a fully aliphatic diol, is a naturally occurring triterpenoid that can be extracted from the bark of birch trees. Due to its rigid pentacyclic structure and biological and pharmacological properties, betulin was studied as a bio-based monomer for preparing synthetic polymers. Here, betulin-based thermoplastics were synthesized using a melt polycondensation procedure with succinic acid, adipic acid, 1,12-dodecanedioic acid, and 1,18-octadecanedioic acid. Successful synthesis for each polymer was confirmed using 1H-NMR spectroscopy. These thermoplastics exhibited weight average molecular weights that ranged from 7,500 g $mol^{-1}$ to 60,300 g mol−1 and displayed glass transition temperatures ranging between 48 °C to 214 °C. Young's moduli and tensile strengths exceeded 600 MPa and 9 MPa, respectively. Additionally, glycerol was used for the formation of crosslinked betulin-based thermosets. These thermosets exhibited storage moduli at 25 °C ranging from 2.0 to 780 MPa. Thermoset glass transition temperatures ranged from 26 °C to 130 °C. Both thermoplastics and thermosets were thermally stable at temperatures greater than 300 °C in both nitrogen and air atmospheres. This work demonstrates the viability of utilizing betulin to prepare polyesters with potentially 100% bio-based content for a variety of applications.

**Synthesis and Properties of Renewable, Aliphatic Polyesters from Thiol-ene and Acyclic Diene Metathesis Polymerization of Alpha, Omega-unsaturated Dienes Derived from Fatty Acids.** Bryan Moser, USDA ARS NCAUR, USA; Benetria Banks, USDA ARS NCAUR, USA; Kenneth Doll, USDA ARS NCAUR, USA

Conventional petrochemical plastics are nonrenewable, proliferate in the environment as waste, are harmful to animals who ingest their residues, and resist microbial degradation. Renewable alternatives that address these deficiencies are thus an important area of current research. A promising approach is the production of biodegradable, aliphatic polyesters from fats and oils that mimic the properties of industrial polyolefins, such as polyethylene, due to the presence of long-chain aliphatic repeat units arising from the fatty acid backbone. We thus report herein the synthesis and properties of renewable, aliphatic polyesters prepared by acyclic diene metathesis (ADMET) and thiol-ene polymerization of alpha, omega-unsaturated monomers derived from 9-decenoic acid (9DA), a terminal fatty acid readily obtained from ethenolysis of oleic acid. Condensation of 9DA with ethylene glycol, 9-decen-1-ol and allyl 9-decenoate provided alpha, omega-dienes suitable for subsequent polymerization. ADMET homopolymerizations were performed in the presence of catalytic Hoveyda-Grubbs second generation catalyst with loss of ethylene to yield an unsaturated polyester. Thiol-ene copolymerizations were conducted photochemically with stoichiometric 1,2-ethanedithiol and 1,3-propanedithiol as linkers along with catalytic 2,2-dimethoxy-2-phenylacetophenone as a photoinitiator to give saturated poly(thioether-ester)s. Both reactions were performed without solvent and proceeded in high yield to the intended polymeric targets. Properties such as molecular weight, glass transition temperature, thermal stability, and green metrics were then determined. In general, the thiol-ene polymers gave higher molecular weights, polydispersities, atom and carbon economies, as well as higher melting, crystallization, and glass transition temperatures relative to their ADMET counterparts. These results and others will be presented and their relevance to industrial applications will be discussed.

**Synthesis of Polyethers, Polyesters, Polyolefins and Polyamides from Renewable Lipids.** Aman Ullah, University of Alberta, Canada

The use of renewable resources in supplementing and/or replacing traditional petrochemical products, through green chemistry, is becoming the focus of research. The utilization of oils can play a primitive role in sustainable development due to their large-scale availability, built-in-functionality, biodegradability, and no net CO2 production. Microwaves, being clean, green, and environmentally friendly, are emerging as an alternative source for product development. Solvent-free conversion of fatty acid methyl esters (FAME's) derived from canola oil and waste cooking oil under microwave irradiation demonstrated dramatically enhanced rates. The microwave-assisted reactions lead to the most valuable terminal olefins with enhanced yields, purities, and dramatic shortening of reaction times. Various monomers/chemicals were prepared in high yield in a very short time. The complete conversions were observed at temperatures as low as 40 ºC within less than five minutes. The products were characterized by GC-MS, GC-FID and NMR. The monomers were separated and polymerized into different polymers including biopolyethers, biopolyesters, biopolyamides and biopolyolefins. The polymers were characterized in detail for their structural, thermal, mechanical, and viscoelastic properties. The ability for complete conversion of oils under solvent-free conditions and synthesis of different biopolymers is undoubtedly an attractive concept from both an academic and an industrial point of view

## Breeding and Biotechnology for Improving Content, Nutritional Quality and Functionality of Plant Proteins—Part 1

*Chairs Phil Kerr, Prairie AquaTech, LLC, USA; Rouf Mian, USDA-ARS, USA; Long Zou (Joe), Bunge North America, USA*

**Environment and Management Effects on Soybean Protein Quantity and Quality.** Seth Naeve, University of Minnesota, USA; Jill Miller-Garvin, University of Minnesota, USA; Ignacio Ciampitti, Kansas State University, USA

Soybean [Glycine max (L.) Merr.] is an important global crop which produces high-quality protein meal and vegetable oil. Soybeans are currently valued in part based on protein concentration that varies spatially within the U.S. due to genetics, crop management, and environmental conditions such as latitude, soil type, length of growing season, and weather patterns. A better understanding of limits to soybean composition will lead to focused breeding and agronomic efforts to improve the value of the crop for end users. Cooler growing conditions in the north and west regions of the U.S. production area tend to result in lower protein soybeans; however, soil types at a smaller geographical scale appear to have a large effect on protein and oil accrual. Soybean management can affect soybean seed composition, but these effects tend to be smaller than the environmental effects noted. Delayed planting tends to reduce oil deposition, and therefore, oil concentration and yield. Crop rotation instead of soybean after soybean increases both seed protein concentration and yield. Nitrogen fertilizer applications can increase protein concentration and/or yield, but effects tend to be small and unpredictable. Other management practices such as no-till, seed treatment, foliar nutrient and fungicide application, and Bradyrhizobium inoculation have shown mixed results on seed composition.

In controlled field experiments, we have shown that the amino acid composition of soybean protein was affected by protein concentration. We found that amino acids essential for the growth of monogastric animals tended to be diluted at high seed protein concentrations by arginine and glutamic acid; this is especially true for the limiting amino acids lysine, cysteine, methionine, and threonine. However, relationships between protein concentration and relative abundance of individual amino acids are affected by specific environmental conditions.

**Optimizing Management for Higher Soybean Seed Protein Concentration.** Anna Locke, USDA-ARS, USA; Kenedy Etone Epie, USDA-ARS, USA

Soybean seed protein, the most economically valuable component of the seed, has decreased in concentration over the years as yield increased. Although soybean seed composition is largely determined by genotype, environmental factors also play a role in determining the concentrations of protein and oil in the seed. While crop breeders develop varieties with higher protein content, farmers are looking to manageable environmental factors as another strategy to increase seed protein concentration. However, there are no management recommendations for increasing seed protein concentration, and even very little information regarding which management decisions have the greatest potential for optimization. The goal of this research was to determine what management practice(s) could be optimized for higher seed protein concentration. Meta-analysis was conducted using published data from fertilization, tillage, irrigation, and row spacing studies, in conjunction with multi-location, multi-genotype field studies. In the meta-dataset from 34 studies containing 1094 pairwise comparisons, nitrogen fertilization, tillage, irrigation, and plant density did not significantly change seed protein concentration, while sulfur fertilization significantly increased seed protein concentration while also increasing yield and oil concentration. In two years of field data from three locations and five genotypes,

sulfur was also the only management practice that had a significant effect on seed protein concentration. This study highlights sulfur fertilization management as a critical area of study for agronomists seeking to improve protein concentration in soybean without loss of yield or oil production.

**Temporal Changes in Metabolism Hold the Key to Carbon Partitioning for a Better Soybean.** Doug Allen, USDA ARS, USA; Timothy Durrett, Kansas State University, USA; Shrikaar Kambhampati, Donald Danforth Plant Science Center, USA; Jose Aznar-Moreno, Kansas State University, USA; Trish Tully, Donald Danforth Plant Science Center, USA; Kristin Bilyeu, Agricultural Research Service, USA; Thiya Mukherjee, Donald Danforth Plant Science Center, USA; Dechassa Duressa, Kansas State University, USA; Jennifer Arp, Donald Danforth Plant Science Center, USA; Kevin Chu, Agricultural Research Service, Donald Danforth Plant Science Center, USA; Stewart Morley, Agricultural Research Service, Donald Danforth Plant Science Center, USA

Soybeans are second only to corn in U.S. agricultural crop revenue. Valued for the highest concentration of protein in a crop seed and paired with significant oil levels that qualify soybeans as an oilseed, this crop is a significant source of food, feed and fuel products. Frequently, reports describe the inverse correlation at maturity between the level of protein and oil; however, the tradeoff is the cumulative effect of resource allocation throughout development which also includes significant partitioning of carbon to carbohydrates such as raffinose family oligosaccharides and cell wall polysaccharides. Oligosaccharides are not digested for energy in monogastric animal diets and carbohydrate polysaccharides are of lesser value than protein or oil. We quantified maternal nutrient provision that is the supply for seed-based metabolism which drops significantly during the later time regime of seed development. This implies that reserves made near the end of development result from re-allocation of existing resources within the seed. We present biochemical evidence that suggests in part the long-observed turnover over lipids late in seed development may contribute in several ways to support biosynthesis of carbohydrates. The production of reserves temporally presents interesting opportunities to alter seed composition for the better and can also result in changes in seed quality by impacting the profile of amino acid concentrations in protein, fatty acid profile in lipids or the relative levels of cell wall polysaccharides and raffinose family oligosaccharides. We will describe the temporal differences that suggest opportunities for engineering that are currently under further investigation.

**Yellow Pea (pisum Sativum l.) Varieties for High Seed Protein Yield in Nebraska.** Dipak Santra, University of Nebraska-Lincoln, USA; Kaustav Majumder, University of Nebraska-Lincoln, USA; Amit Mitra, University of Nebraska-Lincoln, USA; Bijesh Maharjan, University of Nebraska-Lincoln, USA

Pea (Pisum sativum L.) emerges as an important crop for plant-based protein ingredients to the US food industry since it is non-GMO, non-soy, climate-friendly to produce, and easy to blend with any food formulation. People especially the millennial population is preferring plant-based protein because of its human- and environmental health benefits. In the US, traditional pea growing regions are MT, ND, and Pacific North West. Pea is relatively a new crop in Nebraska. Commercial production of field pea with significant acreages started in the state in 2013. Pea acreages in Nebraska increased from a few hundred acres to 35,000 acres in 2020 (max. 75,000 acres in 2017). Can Nebraska play an important role in producing this important plant-protein ingredient crop? The objective of this presentation is to report performances of commercial pea varieties, commonly available in North America, under Nebraska production conditions. Nineteen pea varieties from three leading pea seed companies (Meridian Seeds, Pulse USA, and Valesco Genetics) were tested at three different field locations in western Nebraska (Sidney, Alliance, and Grant) during 2018–2020 following standard production practices including optimal fertilizer for highest productivity. Three years averages of nine site-years data are as follows: Seed yield average was 29 bu/acre (24–33 bu/acre); seed protein % average was 25% (23–27%); average protein yield was 422 lbs/acre (356–506 lbs/acre). Varieties also varied in flowering (June 10–16 with average June 12), 1000 seed weight (208–241g, average 223g), and plant height (17–21 inches with average 19 inches). Nebraskan farmers and seed producers are using this data to select the best pea varieties for the state. We believe that pea acres in Nebraska will increase with the availability of improved varieties and production technology in the future. Nevertheless, this current information is significant for the emerging pea industry in Nebraska.

## Breeding and Biotechnology for Improving Content, Nutritional Quality and Functionality of Plant Proteins— Part 2

*Chairs Rouf Mian, USDA-ARS, USA; Phil Kerr, Prairie AquaTech, LLC, USA; Long Zou (Joe), Bunge North America, USA*

**A New Soybean for Quality Plant Protein.** Kristin Bilyeu, Agricultural Research Service, USA

The importance of soybean is reflected in the worldwide production of over 340 million metric tons in 2018. Soybean value depends on the position on the value chain. Soybean is processed into two main products, the

vegetable oil and a protein-rich meal. While it was originally grown in the US as a forage crop, soybean became the major domestic oilseed crop up to 2005 when US nutrition facts labels were required to include trans fat content. Changes in the food industry reduced demand for commodity soybean oil. Soybean presently derives over two thirds of its value from the meal. Competition from other protein sources put pressure on soybean meal to provide increased value for livestock feed formulations. Out of this scenario is an opportunity for a new soybean that delivers increased value from functional traits for both oil and meal that deliver the highest quality plant protein. Identifying the optimum set of seed composition characteristics that improve the value of soybean while maintaining high yield potential is critical to the development of a new type of soybean. This new soybean represents an opportunity for researchers in areas of vegetable oils and oil byproducts such as lecithin or tocopherols as well as a more neutral flavored high protein meal without undesirable oligosaccharides that can serve as a unique input stock for plant-based protein food research. The high oleic/low linolenic acid oil trait plus the high energy meal trait is a combination that has potential to be the next commodity soybean. Understanding the biological effects of this combination will be important to ensure more functional soybeans can be successfully deployed and utilized throughout the value chain.

**Effect of Enzymatic Hydrolysis on Plant Protein Solubility.** Terrence Dent, Ohio State University, USA; Osvaldo Campanella, The Ohio State University, USA; Farnaz Maleky, The Ohio State University, USA

Enzymatic hydrolysis is a commonly utilized technique in the processing of protein ingredients to improve their functional properties. Several studies have reported improved solubility of plant protein hydrolysates, particularly near the isoelectric point of the unhydrolyzed protein. Review of literature shows unclear methodology in how the hydrolysis is conducted. In some studies, it is explicitly stated that an insoluble fraction is formed during the hydrolysis and removed by centrifugation or filtration. This study aims to better understand the effect of hydrolysis on plant protein solubility by carefully controlling key processing parameters. Protein isolates from soybeans and chickpeas were hydrolyzed using two different commercial enzymes, Flavourzyme and Alcalase. Various processing parameters including hydrolysis time,maintaining constant pH, shearing, and endogenous enzyme inactivation were manipulated and applied in order to test their effect on the solubility of the hydrolyzed protein. After hydrolysis, the degree of hydrolysis was measured using the OPA method and protein solubility was assessed using the Bradford method. The results indicate that overall protein solubility decreased with increasing hydrolysis time and increasing degree of hydrolysis, with different protein-enzyme combinations effecting the overall decrease in solubility. Furthermore, shearing during and after hydolysis and endogenous enzyme inactivation had no effect on the hydrolysate solubility. Loss of hydrolysate solubility compared to unhydrolyzed protein indicates a deviation from commonly published findings, which result from questionable filtration steps which remove insoluble protein and artificially increase the apparent solubility of hydrolyzed proteins.

**Evaluating Accelerated Selection Methods for Breeding High Methionine Soybean Varieties.** Bo Zhang, Virginia Tech, USA; William Singer, Virginia Tech, USA; Rouf Mian, USDA-ARS, USA

Soybean (Glycine max) is an effective food and feed ingredient because its amino acid profile closely matches the needs for humans and livestock. However, deficiencies in sulfur-containing proteogenic amino acids (methionine and cysteine) limit the nutritional value of soybean products. Methionine content is an especially important consideration for soybean-based livestock feed. Historically, breeding solutions for deficient methionine in soybean have been stifled by poor genetic variability and opposing selection pressure. Complex inheritance mechanisms and arduous phenotyping methods also increase cost and time for variety production. Therefore, this research proposes the use of two accelerated selection methods to incorporate into breeding schemes for methionine content: marker-assisted selection and genomic selection. The potential for marker assisted selection was evaluated using a genome-wide association study (GWAS), whereas genomic selection efficacy was measured using genomic best linear unbiased prediction (gBLUP). Several single nucleotide polymorphisms (SNPs) were identified as targets for marker-assisted selection, and promising genomic selection models were constructed using methionine associated SNPs.

**High Protein Soybean Development and Lysine Amino Acid Improvement.** Vincent Pantalone, University of Tennessee, USA

‘‘Most of the value of soybean is due to its high protein meal. However, there is a negative correlation with yield. As a consequence, decades of selection for yield has resulted in lower protein soybeans. Soybeans from U.S. production regions no longer meet the standard of high protein meal ( >47.5% meal protein). Indeed, the trend is for U.S. soybeans to decline to only 45.6% meal protein by 2030. Complementary parentage can lead to breeding high yielding, high protein cultivars. The cultivar ‘Ellis’ with 48% meal protein and high yield is a benefit to farmers and processors alike. Its combination of high yield and high protein gives it a high estimated processor value per acre (EPVA). Ellis produced an EPVA of $765.10 compared with the mean at $698.30. TN15–5007 is new high protein cultivar. In the

USDA Uniform Test, TN15–5007 produced a high yield of 4,136 kg ha-1, which was not significantly different than the mean yield of commercial checks. It had the greatest seed protein in that test and produced an ultra-high 50.5% meal protein. We conducted experiments over multiple locations of recombinant inbred lines to detect quantitative trait loci (QTL) for protein and amino acids. QTL were detected on multiple chromosomes. R2 values were reported as high as 39.1, typically denoting major underlying genes. The larger the LOD score and R2 value, the more confident we are that the QTL will lead to genetic gains in a marker assisted selection program. It is possible that one of more of the QTL represent key enzymes in the amino acid biosynthetic pathway. Further research will enable us to develop superior soybeans with better yield, protein content and quality.

**Improving Soybean Seed Composition with Metabolic Engineering and Genome Editing.** Zhan-Bin Liu, Corteva Agriscience, USA

Soybean is a commodity crop highly valued for its protein and oil contents. Vegetable oil and protein-rich meal are two main processed products for food and feed application. Currently, soybean derives over two thirds of its value from the meal, while the oil contributes to the other one third of its value. Improving both protein and oil content and their compositions in soybean seeds provides an opportunity to create a next generation commodity soybean. Both metabolic engineering and gene editing technology have been used to achieve these goals. Research progress in this area will be discussed in this presentation.

## Biobased Surfactants

*Chairs George Smith, Sasol, USA; Douglas Hayes, University of Tennessee; USA*

**Application of Sophorolipid Technology in Cosmetics.** Glen Lelyn Quan, Saraya Co., Ltd., Japan; Akihiro Fujimoto, Saraya Co., Ltd., Japan; Michiaki Araki, Saraya Co., Ltd., Japan; Naoki Ichiyanagi, Saraya Co., Ltd., Japan; Yoshihiko Hirata, Saraya Co., Ltd., Japan

Sophorolipid is a promising glycolipid biosurfactant which has known characteristics such as excellent detergency and good rinsability, to name a few. Another quality to explore is its gelling property, which can be applied in cosmetics.

Glycerin is commonly used in cosmetics and personal care products as humectant, and comes third after water and fragrance for the most frequently used cosmetic ingredient. However, its liquidity sometimes makes it difficult to be used in other formulations. This investigation focused on the gelation attempt of glycerin using sophorolipid and its application in cosmetics. Investigation on surfactants used in cosmetics including sophorolipid was performed to confirm which can create glycerin gel. From the results, it was confirmed that gel formation of glycerin could be achieved with sophorolipid. The results were further evaluated using scanning electron microscopy, where a densely structured network of glycerin and sophorolipid was confirmed.

Applying this technology in cosmetics, glycerin gel was able to create a thermally irreversible stable glycerin gel network with any oil even without other additives. This sophorolipid technology could help achieve simpler and cleaner cosmetics and personal care products in the future.

**Biobased Surface-active Polymers.** George Smith, Sasol, USA; Alicia Huggett, Sasol, USA; Gabe Ortego, Sasol, USA

Work has been performed using vegetable oil as a feedstock to make water-soluble surface-active polymers for home and personal care applications. By reacting the oil with a polyol, a variety of different surface-active polymers can be prepared. The surface properties on biobased surface active polymers have been measured. In general, biobased surface active polymers show low CMC and surface tension. They show good interfacial tension reduction and detergency in textile cleaning applications. Biobased surface active polymers are good foam stabilizers for liquid dish, shampoo and body wash.

**Formulation Design of High Performance Biosurfactant Based Personal Care Products.** Samiul Amin, Manhattan College, USA

The cosmetic and consumer industrial sector is a high growth industrial sector roughly valued at $532 billion. Due to consumers' increasing awareness on product sustainability, microbially produced biosurfactants are increasingly gaining the interest of the home and personal care industry as potential alternatives for traditional petroleum derived and chemically synthesized surfactants. The future of personal care and detergent products is the elimination of non-biodegradable, environmentally toxic surfactants. Additionally, synthetic polymers utilized extensively in these industries for performance enhancement are being replaced by biodegradable biopolymer alternatives. New sustainable ingredients like chitosan and silk proteins are also showing considerable promise as new high-performance alternatives. However, a move to fully sustainable formulations utilizing novel alternatives will only be successful if the performance criteria such as rheology and surface activity matches or surpasses that obtained from traditional surfactants and polymers. This requires engineering the microstructure and interactions in these formulations and a re-establishment of the microstructure-property-performance linkages in

these complex fluid-based products. In this talk this approach of engineering the microstructure and interactions for effective product formulation design will be illustrated for a range of sustainable and biodegradable alternatives and their combinations, namely, glycolipids, silk proteins and chitosan. The physico-chemical insights required for establishing the formulation design rules were generated utilizing a range of advanced characterization techniques. These include force tensiometry, interfacial rheology, diffusing wave spectroscopy (DWS), raman spectroscopy, dynamic light scattering etc. As will be highlighted in this talk, the combined utilization of these techniques especially diffusing wave spectroscopy, optical microrheology and Raman spectroscopy offers new insights into the mechanisms and pathways of the self-assembly process and understanding of the driving forces associated with changes in viscosity and viscoelasticity in these complex fluids-based products.

**Production of Fatty Alcohols from Biomass-derived Chemicals Using Chemo-catalytic and Bio-catalytic Pathways.** Dimitris Collias, Procter & Gamble, USA; Stephen del Cardayre, Sustainability and Health, USA

Fatty alcohols can be produced via either a chemo–catalytic or a bio-catalytic pathway from biomass-derived chemicals, such as, sugars, methyl furfural, acids, ketones, and aldehydes. After a detailed description of the two pathways, we will compare them in terms of process conditions and estimated economics.

In the chemo-catalytic pathway, a stepwise process was developed that first builds the carbon skeleton from biomass-derived chemicals to a targeted structure and length, which is then followed by a selective removal of all but the acid or ester group. This process is exemplified in the making of methyl-dodecanoate from a carbon skeleton containing furan, alkene, ketone, and ester functionalities. The catalyst selection for the selective removal step will be described in detail. Some catalysts were able to effectively catalyze this reaction under specific conditions (temperature and space velocity) without over-reacting the acid or ester product to alkanes.

In the bio-catalytic pathway, sugars were selectively converted in a single step fermentation to C12 and C14 fatty alcohols. The conversion was catalyzed by a genetically engineered strain of E. coli, whose natural fatty acid biosynthesis pathway was highly upregulated and diverted into a novel fatty alcohol biosynthesis pathway. The novel pathway was composed of a thioesterase, fatty acid carboxylic acid reductase, and aldehyde reductase. The fermentation process approached 80% of theoretical yield, was successfully scaled in a 132,000 L demonstration scale bioreactor, and was included in a Presidential Green Chemistry award made to LS9 in 2010.

**U.S. Soy: High Oleic Soybean Surfactants.** Dwight Rust, Omni Tech International, LTD, USA

The U.S. farmer-led United Soybean Board is committed to bringing useful information to companies to fulfill sustainability goals and promote products utilizing U.S.-grown soy. Renewable by nature, U.S. soy can be used as a raw material in a diverse group of surfactants. Funded by the United Soybean Board, Battelle Memorial Institute has developed a platform technology to produce a unique class of surfactants for use in laundry and cleaning applications.

This presentation will discuss these surfactants which are based on high oleic soybean oil as conventional soybean oil cannot be used due to rapid side reactions leading to undesirable by-products. The chemistry utilized produces highly water miscible and water stable surfactants with the ability to control bio-content, functional groups (such as chelators), and ultimately vary the hydrophilic-lipophilic balance (HLB) to improve performance. Replacement of nonionic and anionic surfactants in a standard laundry formulation has shown improvements in targeted stain removal such as coffee and make-up stains.

## Biotechnology Poster Session

*Poster Chairs Todd Underiner, P&G, USA; Sarah Willett, Kerry, USA*

**2-Hexadecynoic acid targets Methicillin-Resistant Staphylococcus aureus plasma membrane and fatty acid synthesis.** Giancarlo Casillas-Vargas, Natural Sciences, Inter American University of Puerto Rico-Metropolitan Campus, USA; Carlimar Ocasio-Malave, Inter American University of Puerto Rico-Metropolitan Campus, USA; Nestor Carballeira, University of Puerto Rico, Puerto Rico; David Sanabria-Ríos, Inter American University of Puerto Rico-Metropolitan Campus, USA

Methicillin-resistant Staphylococcus aureus (MRSA) is part of the wave of antibiotic-resistant bacteria that have emerged in the last decades, and its infections had become a challenge to treat. These strains can cause skin and wound infections, threatening life pneumonia, and bloodstream contamination that could trigger sepsis and death. Fatty acids (FA) exhibit cytotoxic activity against a wide range of bacteria and represent alternative treatment to traditional antibiotics. In previous studies, 2-hexadecynoic acid (2-HDA) antibacterial activity against MRSA isolates has been tested. However, relatively little is known about the underlying mechanism of antibacterial action of 2-HDA. Therefore, this study aims to determine the mechanism of action of this unsaturated fatty acid (uFA) against a clinical isolate of MRSA (CIMRSA). 2-HDA-treated MRSA showed fluorescein permeabilization like linoleic acid

(LA), a well-known membrane disruptor agent, whereas palmitic acid (PA) did not show any effect. Additionally, by detecting 260-nm released absorbing material from bacteria such as DNA and RNA, we found higher genetic material concentrations in treated cells' extracellular space. LA showed to be more effective in the first 60 min, whereas 2-HDA resulted more efficacious when reached 80 min. Moreover, a quantitative real-time PCR (RT-qPCR) assay showed that 2-HDA, like LA, suppressed the expression of fabI gene that encodes enoyl-acyl carrier protein reductase (FabI), an essential protein of the bacterial membrane fatty acids synthesis. Our findings suggest that 2-HDA targets the FA synthesis and is a promising membrane disruptor agent synthesis, which could represent a new option to treat emerging antibiotic-resistant S. aureus.

**Analysis of the Antibacterial Activity Differences for the Final Products between Synchronous and Nonsynchronous Enzymatic hydrolysis Process on Collagen and Evaluation of the Scale-up Production Feasibility.** Shu-Wei Chang, Dayeh University, Taiwan (Republic of China); Yun-Jane Lin, GeneTex, Inc., Taiwan (Republic of China)

Justification: In recent years, a variety of biologically active peptides (small protein fragments) can produce physiological regulation functions such as hormonal effect, antibacterial and anti-tumor etc., and their bioavailability is much higher than that of proteins and amino acids.

Objective: We planned to use different enzyme mixture or combination to hydrolyze different collagen materials (pig skin/fish skin) by using synchronous or non-synchronous (stepwise addition) approaches, to compare the antibacterial activity differences of the final products.

Methods: The pig/fish skin collagen is dissolved in 50–500 ml of distilled water, heated at 90°C for five minutes, and the hydrolysis reaction is carried out under the most suitable conditions for different type of enzyme as show in Table 1.

Table 1. The optimal reaction conditions for different commercial protease

| Reaction conditions | Enzyme Types | | | |
|---|---|---|---|---|
| | Pepsin | Papain | Trypsin | Bromelain |
| Reaction Temperature | 37 °C | 55 °C | 50 °C | 45 °C |
| Reaction pH | 2.0 | 7.0 | 8.7 | 4.5 |
| Reaction time | 5 h | 5 h | 8 h | 4 h |

The difference in antibacterial activity is compared by calculating the inhibition rate according to the diameter of the inhibition ring.

Results: For antibacterial activity, only the asynchronous hydrolysates (two-stage addition of bromelain/trypsin mixture as biocatalyst) can be reached 66% inhibition rate on Escherichia coli, although it is lower than that obtained by phenoxyethanol (87%). The optimal hydrolysis conditions of pig skin collagen (trypsin mixed with bromelin were added asynchronously) has been successfully performed in a scale-up reaction (500 mL), suggested the high feasibility of this process for commercial scale-up production in the future.

Significance of your research to the AOCS membership: This experiment found that a functional method (non-synchronous addition of enzyme mixture) with high and diverse biological activities. It shows that it has high development potential and can be widely used in health, food, medicine, cosmetics and other industries.

**Application of co-cultures of fungal mycelium during solid-state fermentation of canola meal for potential feed application.** Ahmad Alhomodi, South Dakota State University, USA; Andrea Zavadil, South Dakota State University, USA; Mark Berhow, USDA ARS NCAUR, USA; William Gibbons, South Dakota State University, USA

Canola meal (CM) is a by-product of canola seed oil extraction and contains up to 50% protein on a dry basis. Despite CM being the high protein commodity with well-balanced amino acid composition, it remains one of the more underutilized by-products of the oil processing industry. Only a fraction of produced CM goes into animal feeds as presence of antinutrients such as phenolics, glucosinolates (GLS), phytates, and high fiber content makes this product undesirable. Mixed microbial cultures have been used successfully in commercial scale, fermentations such as in wastewater treatment plants, for soil remediation, in biogas production, and in the production of traditional foods such as cheese, yoghurt, pickles and in alcoholic beverage production. Thus, in this study, the mono, bi, and tri-cultivation of Aureobasidium pullulans, Neurospora crassa and Trichoderma reesei in solid state fermentation were applied to improve the nutritional value of hexane extracted canola meal along with the reduction of antinational factors for animal feed applications. The data showed that fungal cultivation had positive effects on the level of protein, fiber and, glucosinolates (GLS). Monoculture of N. crassa exhibited the highest protein level of 49%. The combination of A. pullulans and N. crassa provided the highest reduction of crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) by 21.9%, 1.7% and 9.1% respectively. Biculture of A. pullulans and T. reesei resulted in the best GLS reduction by 81.3%. These results indicate that each fungal strain owns different enzymatic ability and selectively can work with other fungi in synergistic relationship for better fungal conversion of canola meal.

**Impacts of discoloration on the antibacterial and antifungal bioactivities of a porcine cruor hydrolysate.** Aurore Cournoyer, Laval University, Canada; Zain Sanchez Reinoso, Laval University, Canada; Laila Ben Said, Laval University, Canada; Jacinthe Thibodeau, Laval University, Canada; Sergey Mikhaylin, Laval University, Canada; Ismail Fliss, Laval University, Canada; Laurent Bazinet, Laval University, Canada

Porcine blood is a major by-product from slaughterhouses and has a high potential for valorization. Indeed, the solid part of blood, designated as cruor, has a high proportion of proteins: 90 % of hemoglobin. By conducting enzymatic hydrolysis of porcine hemoglobin, using pepsin, a wide variety of peptides can be obtained. They are known to include bioactive peptides, such as antimicrobials. Many studies have been performed on hydrolysates from bovine hemoglobin but few on porcine hemoglobin. The color of the cruor caused by the presence of haem, can be removed by a discoloration procedure, where haem is precipitated. This step increases its potential for applications in food and more specifically white meat. The consequences of this additional step were evaluated on the antibacterial and antifungal activities. The results showed that, after precipitation of haem, the recovered peptides in raw hydrolysate and discolored one were different. Some peptides disappeared during this step. In addition, both hydrolysates showed antifungal activities but no antibacterial activity. The absence of antibacterial activity was due to the short duration of hydrolysis and the concentration of peptides. The degree of hydrolysis increases with time and, therefore, the length and concentration of peptides differ. However, in terms of antifungal activities, significant differences were observed between the two hydrolysates, along with the discoloration pellet. The raw hydrolysate had an antifungal activity up to 10 times higher than with the discolored one. The discoloration causes the loss and reduction of specific peptides, also found in the bioactive pellet. These precipitated peptides, which sequences were identified, would be responsible for the antifungal activity. To our knowledge, it is the first time that sequences of antifungal peptides from porcine cruor hydrolysate were proposed. The next step is the synthesis of these potential active peptides and to test them individually or in mixture.

**Improved lipid extraction method for the analysis of fatty acid distribution in TAG of infant formula.** Yomi Watanabe, Osaka Research Institute of Industrial Science & Technology, Japan; Hirofumi Sato, O R I S T, Japan; Masahiro Muraoka, OIT, USA

Röse-Gottlieb method is a conventional and common method to extract lipids from milk and infant formulas. Previously we have reported that lipids, consists mainly of TAG (~98%) were successfully extracted from most infant formulas in powder form that purchased locally, and the fatty acid distribution within the resulting TAG including polyunsaturated fatty acids was analyzed by Joint JOCS/AOCS Official Method Ch 3a-2019 (Y. Watanabe et al. Milk Science, 69, 63–70, 2020).

However, one liquid formula showed low extraction yield (~70%). After several attempts to improve the yield, it was found that adsorption of liquid formula to silica gel increased the extraction yield more than 10% 15 g of silica gel 60 was added to 10 mL of liquid formula, treated under reduced pressure using rotary evaporator to remove water. Lipids were then extracted from the formula-adsorbed silica gel by 150 mL of 1:1 mixture of petroleum ether and diethyl ether. This extraction method has an advantage that the treatment of samples with ammonium water before the ether extraction step could be skipped. It was estimated that adsorption on silica gel contributed to break emulsions in the formula efficiently and improved the extraction yield.

**Integrated bioinformatics approach for identification of bioactive peptides from orange-footed (Cucumaria frondosa) sea cucumber hydrolysate by-products.** Tharindu Senadheera, Memorial University of Newfoundland, Canada; Deepika Dave, Memorial University of Newfoundland, Canada; Fereidoon Shahidi, Memorial University of Newfoundland, Canada

Orange-footed sea cucumber (Cucumaria frondosa) is the most common holothurian in Northwest Atlantic waters. The body wall of sea cucumber is the major marketable portion. The remaining tissues representing up to 50% of the body mass, including viscera, are discarded as processing waste. The production of protein hydrolysates from sea cucumber viscera is an effective approach for adding value to these by-products. Alcalase and Flavourzyme assisted hydrolysis was conducted to process sea cucumber viscera. The protein hydrolysates so produced were assessed for their potential antioxidant activities. In vitro radical scavenging activity assays using DPPH and hydroxyl radicals were performed, and the efficacy of inhibiting beta-carotene bleaching was determined using an oil-in-water emulsion model system. The protein hydrolysates were further analyzed for their amino acid sequences using liquid chromatography-mass spectrometry (LC-MS/MS). The identified peptides were virtually screened for identification of antioxidative peptides. In silico analyses predicted that sea cucumber viscera can be utilized as a precursor for generating the antioxidative peptides with potential for use in functional foods for human health promotion. Thus, integrating the emerging bioinformatics tools provide a plethora of information for validating the findings in wet lab synthesis. The current study also demonstrates a possible pathway for utilization of sea cucumber processing discards.

**Role of SOD1, GPX1 and CATvariants with associated pattern of haplotypes and their effect on differential expression in the susceptibility of diabetic cataract.** Sanober Kafeel, University of Karachi, Pakistan; Syeda Nuzhat Nawab, University of Karachi, Pakistan; Sanober Kafeel, University of Karachi, Pakistan

Justification: The occurrence of cataract is higher in diabetic patients due to the overproduction of reactive metabolites that develops oxidative stress. The primary antioxidant enzymes SOD1, CAT and GPX1 play a significant role in the dissociation of reactive metabolites in the lens compartment and provides protection.

Objective: The objective was to investigate the role of SOD1 50 bp Indel, CAT -262 C/T and GPX1 C/T polymorphisms in modulation of differential expression in the pathogenesis of diabetic cataract.

Methods: It was a case control study comprised of n=680 blood samples divided into four groups (n=170 each). Lenses of diabetic cataract and senile cataract subjects were also collected. Genotyping of SOD1 50 bp Indel, CAT -262 C/T and GPX1 C/T polymorphisms were performed using PCR. The estimation of SOD1, CAT and GPX1 levels were carried out by ELISA protocol. Western Blotting was done to validate the differential expression of GPX1 enzyme in senile cataract and diabetic cataract lenses. The data was analyzed using statistical and bioinformatics' software.

Results: Results indicated the significant role of mutant T allele of GPX1 C/T polymorphism in the susceptibility of diabetic cataract ($p < 0.001$). Linkage disequilibrium analysis revealed the pattern of co-inheritance and linkage of haplotypic markers 7 and 8 in diabetic cataract patients ($D' = 1$, LOD >2). The serum levels of SOD1, GPX1 and CAT enzymes were significantly lower in diabetic cataract patients ($p < 0.001$). The expression of GPX1 enzyme was found to be $-2.7$ fold reduced in diabetic cataract as compared to senile cataract group.

Significance: The findings suggested the significant predisposing role of GPX1 C/T polymorphism in reducing the GPX1 enzyme expression which could be associated with the progression of diabetic cataract. Therefore, it can serve as a potential antioxidant marker to develop strategies against the early onset of cataract in type 2 diabetic patients.

**Study on the Effect of Enzyme Assisted Method on Extraction Efficiency of Polysaccharides from Seaweed.** Shu-Wei Chang, Dayeh University, Taiwan (Republic of China); Tsai-Wei Huang, Dayeh University, Taiwan (Republic of China)

Justification: Seaweed is rich in polysaccharides, protein, lipid, pigment and low-molecule substance that enhances immunity and anti-cancer activity. This kind of marine polysaccharides are produced by enzymes and have multiple special oligosaccharide components, which can improve macrophage cell activity, whiten and moisturize, anti-oxidation, lower blood lipids and blood purification, and other special physiological functions.

Objective: This study intends to explore the effect of enzyme assistance on the extraction rate of seaweed polysaccharides, and to screen out the types of seaweeds with high extraction rates and their optimal extraction conditions.

Methods: In this study, we planned to take 3–4 different types of seaweeds, including kelp, laver, hairpin, and kelp sprouts, and extract them with enzymes or without enzymes, to compare the differences in their polysaccharide content.

Results: Preliminary experimental results show that adding enzyme-assisted extraction at 37°C can effectively increase the extraction amount of seaweed polysaccharides. Among them, kelp buds have the best effect, with a total sugar content of $2.01 \pm 0.05$ mg/mL, which is about 4-fold higher than that obtained by without enzymes ($0.43 \pm 0.05$ mg/mL).

Significance of your research to the AOCS membership: While the world's population is gradually increasing, algae have gradually become another major source of food for development, so it has great development potential.

**Tailoring Camelina sativa seed fatty acid composition via Fast Neutron mutagenesis.** Xinjie Liu, University of Saskatchewan, Canada; Xinjie Liu, Helen Lui, Dan Hupka, Xiao Qiu and Mark A Smith.

Please see Biotechnology Division Student ePoster Pitch Competition category for full abstract

***Abstract for the following presentation was not provided.***

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**Recent Achievements in Biocatalytic Reactions for Oleochemistry.** Uwe Bornscheuer, Institute of Biochemistry, Germany

## Edible Applications Technology

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*Division Vice Chair: Kaustuv Bhattacharya, IFF, Denmark*

## Edible Applications Technology Division Student ePoster Pitch Competition

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*Judges Amanda Wright, University of Guelph, Canada; Filip Van Bockstaele, Ghent University, Belgium; Kaustuv Bhattacharya, IFF, Denmark*

**Effect of interfacial composition on the rheological and digestion behaviour of emulsions.** Kunal Kadiya, University of Saskatchewan, Canada; Manisha Sharma, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

The rheological behaviour of emulsion can be tuned either through random jamming of droplets or the formation of a network of aggregated droplets leading to the structure formation in many food applications. The random jamming in nanoemulsion, at relatively low oil concentration, could be induced by decreasing the droplet size to nanoscale and increasing the interfacial shell-layer thickness. In this work, the interfacial shell-layer thickness was modulated by electrostatic deposition of positively charged chitosan (degree of deacetylation, DDA50% and DDA93%) on negatively charged Citrem (citric acid esters of mono-and-diglycerides)-stabilized nanodroplets. The aim was to determine the influence of interfacial shell-layer composition on the rheology and digestibility of emulsions at different concentrations (0–0.25wt%) of chitosans. With an increase in chitosan concentration and DDA, the negative charge of Citrem-stabilized droplets shifted to zero at 0.075wt% and reached a maximum at 0.2–0.25wt% chitosan. Correspondingly, the droplet aggregate size increased initially due to bridging flocculation till 0.1wt% and started decreasing at surface saturation around 0.2wt% chitosan. The gel strength of emulsions showed a similar trend with a significant increase up to 0.1wt% chitosan and decreasing thereafter and forming a stable viscoelastic gel at 0.2wt%. However, a further increase in chitosan concentration (0.25wt%) led to weak gels. The repulsive gelation in bilayer emulsions was attributed to an increase in the interfacial thickness by chitosan due to the formation of a strong steric interfacial layer on the Citrem-stabilized nanodroplet. The increase in shell-layer thickness was found to be a controlling factor for lipid digestion, where completely covered droplets (0.25wt% chitosan) showed lower lipolysis compared to zero or lower concentration of chitosan. Such a change in the interfacial composition, to produce bilayer emulsions with tailored rheological and digestion properties, can be used as an attractive option to lower fat and deliver bioactives in emulsion gel-based foods.

**Multi-scale approach to study crystallization behavior of cocoa butter-copra oil blends.** Julie Bloquet-Maurras, Centre de Recherche Paul Pascal, UMR 5031, Université de Bordeaux, CNRS, France; Mathilde Bayard, SOREDAB, France; Véronique Schmitt, Centre de Recherche Paul Pascal, UMR 5031, Université, de Bordeaux, CNRS, France

The aim of this study was to characterize the changes induced in the crystallization of cocoa butter by the addition of different proportions of copra oil. To do so, cocoa butter (CB) and copra oil (CO) were blended in binary systems. Several fat blends with different ratios of CB/CO were crystallized at 4°C and then characterized using various techniques. Phase behavior diagrams were first constructed from the nuclear magnetic resonance results. A multi-scale approach was used to study blends' physical properties such as microstructure and textural properties. The molecular diversity within the fat system was investigated. The microstructure of the fats was determined using a polarized light microscope. Textural properties were examined by rheological measurements at high deformation, using cone penetrometer. Solid fat content (SFC) profiles and isosolid diagrams of CB/CO mixtures showed eutectic formation, indicating a softening of cocoa butter by copra oil addition. The greatest extent of incompatibility occurs at the 60% copra oil addition level in 40% cocoa butter at 20°C. Crystal morphology and network's structure of blends were different as a function of the fat composition. Considering the textural properties, blends with high copra oil contents showed lower hardness in accordance with lower SFC eutectic effect.

**Protein-ligand docking as a tool to predict properties and performance of plant-protein based bioplastics films.** Kristen Patnode, North Dakota State University, USA; Sara Johnson, Winona State University, USA; Andriy Voronov, North Dakota State University, USA; Bakhtiyor Rasulev, North Dakota State University, USA; Zoriana Demchuk, Oak Ridge National Lab, USA

Use of computational models to predict experimental outcomes within food packaging is a new approach and can serve to expedite advances in the field. Studies dedicated to bioplastic film formation have been performed for more than a century. By utilizing computational models, the amount of time and resources required in the wet-lab can be reduced, providing an economically effective means to further develop bio-based food packaging alternatives.

This study applies a combined computational and experimental approach, while focusing on plant protein-based films, which are emerging at the forefront of functional food trends. Protein-ligand docking approach was applied to model the intermolecular (non-covalent) interactions of select renewable additives with plant proteins, to demonstrate the effect of the incorporated modifiers on properties of protein-based films. To support the molecular docking, we performed a set of experiments and successfully prepared films based on soy protein and zein protein which exhibit promising physical and mechanical behavior. By incorporation of natural plasticizers (glycerol and sorbitol) and reinforcement agents (micro-fibrillated cellulose) into the protein systems, films with more advanced mechanical properties and enhanced surface hydrophobicity were prepared, which confirmed the initial computational evaluations. In result, we found that computational

protein-ligand docking approach can be used as an effective and accurate method in estimating the physical and mechanical properties of a film upon incorporation of modifiers into the plant-protein based system.

**Relationship Between Butter Physical Properties and Water Loss in a Laminated Pastry Model System.** Annalisa Jones, Utah State University; USA; Silvana Martini, Utah State University, USA

Butter is commonly used to produce laminated pastry since it provides ideal flavor and texture in the final product. However, during the processing of laminated pastry the butter structure can break due to shear stress, and water present in the form of a water-in-oil emulsion can be released from the system causing less flakiness in the dough. There is a need to unveil the physical properties that drive water loss in butter during lamination. Therefore, the objective of this study is to characterize the physical properties of 10 commercial butter products and correlate these properties to water loss during lamination. Samples were purchased from a local store and stored at 5°C for 24 h prior to testing. The physical properties evaluated include solid fat content (SFC), water content, water droplet size, viscoelastic behavior, melting profile, hardness, and water loss. A negative correlation was identified between water loss and SFC at temperatures of 30°C ($p=0.035$, $r=-0.685$) and 35°C ($p=0.015$, $r=-0.758$). Melting profiles of the samples were characterized by two melting peaks identifying medium-melting-fraction (MMF) and high-melting-fraction (HMF) triacylglycerols. The MMF enthalpy calculated from the melting curve was negatively correlated with water loss ($p=0.001$, $r=-0.915$). The HMF onset temperature ($p=0.002$, $r=-0.879$) and peak temperature ($p=0.006$, $r=-0.818$) also had a negative correlation with water loss. Finally, water loss was negatively correlated to hardness measured at 5°C ($p=0.017$, $r=-0.745$). None of the other physical properties showed a significant correlation with water loss ($p > 0.05$). Overall, this study shows that water loss can be reduced by formulating butters with high SFC at 30 and 35°C, high enthalpy of the MMF melting peak, high onset and peak temperature of the HMF peak and by increasing the hardness.

**Viscoelastic improvement of water-in-oil emulsions for food and related applications.** Maria Romero-Pena, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

In this study, the increase in water content was investigated to improve the viscoelastic behaviour of water-in-canola oil (W/O) emulsions. Six sets of emulsions were prepared with high-pressure homogenization at > 60 ºC using different water fractions (20–50 wt%) containing 1.5 wt% LMP, and the oil phase containing glycerol monooleate (GMO) having same water-GMO ratio. All emulsions contained the identical concentration of hydrogenated soybean oil (HSO) in the oil phase (7 wt %) to structure the W/O emulsions. All LMP-based emulsions remained stable after 30 days at 4°C, with no phase separation and flow behaviour than the control emulsions without LMP. Higher emulsion stability was observed with 40 and 50 wt% water than the emulsions with lower water content. All emulsions increased storage moduli proportional to water content rise without significant change between days 1 and 30, except for LMP-emulsions with 50 wt% water content. Water content augments the cluster size as more water droplets were packing together, forming a network and increasing gel strength. LMP at the interface also allowed the water droplets to weakly flocculated, preventing coalescence. Although all LMP-emulsions kept $G' \geq G''$ showing elastic behaviour, control emulsions showed $G'' \geq G'$ indicating viscous behaviour, suggesting that the presence of LMP is critical for droplet stability and emulsion gelation. Based on the appearance of bulk or emulsified water crystallization peak in differential scanning calorimetry (DSC). LMP-emulsions were completely stable at low water content (20 wt%) while partially stable at higher water content (30– 0 wt%). However, control emulsions showed only bulk water peak, indicating unstable emulsions. Rheology, DSC, and emulsion stability test showed that increasing water droplet network and LMP interaction at the interface enhanced the stability and elasticity of W/O emulsions, which could lower fat content in spread foods products.

## Fundamentals of Fat Crystallization I—Crystallization

*Chairs Alejandro Marangoni, University of Guelph, Canada; Eckhard Flöter, Technical University Berlin, Germany*

**A Molecular View of Crystallization Process of Triglycerides.** Giuseppe Milano, Università Di Salerno, Italy; Alejandro Marangoni, University of Guelph, Canada; Gianfranco Mazzanti, Dalhousie University, Canada; Dalhousie University, Canada; Dérick Rousseau, Ryerson University, Toronto; Canada

In my talk I will show some results related to coarse-grained and atomistic models of triglycerides (TGA) focused on their crystallization behavior. In particular, simulations of a coarse-grained model developed to describe tridecanoin liquid–solid phase transitions have been used to describe at a detailed molecular level the possible mechanism of nucleation of TGA. [1] Using reverse mapping techniques an atomic description of the same mechanism is provided. [2] The mechanism of nucleation of mixtures of different triglycerides has been studied using the same coarse-grained model. In particular, a well characterized mixture of tripalmitine (PPP) and tristearine (SSS) TGAs has been considered. [3] On the basis of this study further investigations related to

the molecular nature of the eutectic behavior of SSS/PPP mixtures will be presented.[4]

References
[1] Development of Coarse-Grained Model for Simulations of Tridecanoin Liquid-Solid Phase Transitions Antonio Brasiello, Silvestro Crescitelli and Giuseppe MilanoPhys. Chem. Chem. Phys 13, 16618, (2011)
[2] A Multiscale Approach to Triglycerides Simulations: From Atomistic to Coarse-Grained Models and Back Antonio Brasiello, Silvestro Crescitelli and Giuseppe MilanoFaraday Discussions, 158, 479, (2012)
[3] Coarse-Grained Modeling of Triglycerides Crystallization: A Molecular Insight into Tripalmitin Tristearin Binary Mixtures by Molecular Dynamics Simulations Pizzirusso, Antonio; Brasiello, Antonio; De Nicola, Antonio; Marangoni, Alejandro; Milano, Giuseppe Journal of Physics D: Special Issue Article Physics of Food 2015, 48 494004
[4] Molecular Insights into the Eutectic Tripalmitin/Tristearin Binary System Antonio Pizzirusso**,** Edmund D. Co, Fernanda Peyronel**,** Alejandro G. Marangoni and Giuseppe Milano Journal of American Chemical Society 2018 140 (39), pp 12405–12414

**Modeling Triglyceride Pure Component Properties by a Configurational Approach.** Julia Seilert, Technical University of Berlin, Germany; Eckhard Flöter, Technical University Berlin, Germany

The successful description of the phase behavior of triglyceride mixtures is crucial to understand complex phenomena in fat-based products. This complex objective is based on reliable predictions of pure component properties and the non-ideal mixing in liquid- and solid-phases. In this contribution, a new model to predict the thermodynamic properties of pure triglycerides, namely enthalpy of fusion and melting temperature, is presented. Different contributions to the thermodynamic properties could be expressed by means of repetitive structural attributes of pure triglycerides deduced from molecular structures. Carefully formulated configurational and geometrical simplifications enabled to attribute physical meaning to most of the parameters. Overall, the number of adjustable parameters was successfully minimized to less than half compared to a well-established model. A comparison of both models revealed that the new model surpasses the reference model considering desirable prediction quality, thermodynamical consistency, and the number of adjustable parameters in order to do so.

**Phase Behavior and Polymorphism of Saturated and Unsaturated Phytosterol Esters.** Eva Daels, KU Leuven, Belgium; Imogen Foubert, KU Leuven Kulak, Research Unit Food & Lipids, Belgium; Zheng Guo, Aarhus University, Denmark; Wim Thielemans, KU Leuven, Belgium; Bart Goderis, KU Leuven, USA

Phytosterols are plant-based compounds which have a blood cholesterol lowering effect. In the form of phytosterol esters they can be used as an ingredient in functional food formulations. Only few publications describe the melting behavior of different types of pure phytosterol esters. However, difficulties arise when trying to compare and use the reported data since it is unclear how the melting temperatures were extracted from the DSC melting curves and the purity of the phytosterol esters used in the different studies is greatly divergent ranging from 8.5–99%. Furthermore, there is currently no X-ray based crystallographic information available on pure phytosterol esters.

This study investigated the influence of chain length and degree of unsaturation of the fatty acid esterified to β-sitosterol on the crystallization and melting behavior of the β-sitosteryl esters by using DSC. Insight in the microstructure was obtained by using polarized light microscopy and the crystal structure of the β-sitosteryl esters was examined for the first time with time-resolved synchrotron X-ray diffraction. The β-sitosteryl esters containing different types of fatty acids (stearic, palmitic, lauric, oleic and linoleic acid) were synthesized by enzymatic esterification.

The results showed that the melting temperature and enthalpy of the β-sitosteryl esters increased with an increasing fatty acid chain length while they decreased with an increasing degree of unsaturation. Furthermore, it was demonstrated that saturated β-sitosteryl esters and β-sitosteryl oleate formed a bilayer crystal structure. The lamellar spacings of the bilayer structure decreased with decreasing fatty acid chain length and with an increasing degree in unsaturation. Only the unsaturated β-sitosteryl esters formed a liquid crystalline phase during cooling.

**Revisiting the Hildebrand and Van't Hoff Approaches for the Prediction of Sfc-temperature Profiles from Triglyceride Molecular Composition and Thermal Properties in Consideration of Cooperativity of Melting Transitions.** Alejandro Marangoni, University of Guelph, Canada

The prediction of SFC from TAG composition, heat of melting and melting temperature has been a long-standing problem in the field of fat crystallization and structure. Some of the earlier attempts at this included empirical models and statistical correlations. The use of the Hildebrand approach was superseded by approaches such as Wesdorp's, which was based on the concept of chemical potential and one and two-way interactions. However, the exponential growth in complexity due these interactions for multicomponent systems and the general lack of data have made this approach only partially successful and remains difficult to implement. Here we propose a two-stage approach where the Hildebrand equation is used to first calculate the melting point depression of a TAG in a mixture of TAGs, and followed by a van't Hoff approach to predict the solid mass fraction as a function of temperature.

However, we have included the concept of melting cooperativity in the model to address possible non-idealities and interactions instead of interaction parameters, even if the treatment assumes ideality of mixtures. The functions derived up to seven components are powerful and very closely predict the melting behavior of several fats. The challenge, however, still remains the lack of knowledge of the exact polymorphic form of each TAG in a mixture since this defines the thermal parameters used in teh calculation. However, one can carry out a Monte Carlo simulation and predict all outcomes. The principle of cooperativity is approached from a geometrical perspective related to a proposed molecular mechanism for lamellae thermal breakdown. Here we will revisit this important, old, and yet still open problem in the field of fat crystallization. This approach is easily implemented on an excel spreadsheet with a look up table.

**Using USAXS to Predict the Under-tempered Chocolate Microstructure.** Fernanda Peyronel, University of Guelph, Canada; David Pink, St Francis Xavier University, Canada

This work compares the microstructure in 70% dark chocolate samples using the ultra-small angle X-ray scattering (USAXS) technique. The chocolates were crystalized using the under- and well-tempered protocols. It was observed that under- and well-tempered chocolates exhibited differences in the q-region ~2 x 10–5 Å-1 < q < ~1.5 x 10–4 Å-1, which correspond to spatial length scales from 32 μm to 3.2μm. The differences are manifested in the mass fractal dimension values, D, obtained when the USAXS data were fitted using the Unified Fit model. The length scale at which these differences were observed coincides with that detectable in the oral cavity. This work proposes that D = 2.1 characterizes under-tempered, dark chocolates, while D = 2.3 characterizes well-tempered, dark chocolates. This work also presents a simple model that describes the disintegration of those aggregates formed by the basic scattering units for under- and well-tempered chocolate. The model proposes that aggregates formed in under-tempered chocolate persist after the bulk chocolate has melted and can be perceived as grittiness. The model also suggests that the aggregates for well-tempered chocolate melt at the same or lower temperatures than the bulk chocolate's melting temperature; hence no grittiness is perceived.

## Fundamentals of Fat Crystallization II—Oleogelation

*Chairs Eckhard Flöter, Technical University Berlin, Germany; Alejandro Marangoni, University of Guelph, Canada*

**Edible Oil Structuring Through Molecular Engineering.** George John, City College of New York (CUNY), USA

Promotion of public health by delivering healthy food is a subject of research with national importance. Fats are among the most vital macronutrients in our diet as they could influence the mouth feel and organoleptic properties of the foods. New regulations have brought new challenges in structuring vegetable oils without chemical modification. This led to the development of other alternatives and, among them, ''oleogels'', a potential strategy that could form bulk fats via physical structuring of vegetable oils. The crystalline composition of oil-based formulations greatly impacts the aesthetic and textural properties of gel and cream formulations. This talk discusses the molecular engineering approach through self-assembly protocols to develop healthy and amphiphilic oil-structuring agents for food and personal care products. By conjugating active substrates to develop an array of molecular gelators from biomass. Chosen precursors for oil gelling amphiphiles (medium chain fatty acids and non-reducing sugars) are FDA approved and GRAS materials; hence the amphiphiles are deemed to be non-toxic and exhibited high cell viability at concentration 50 μg/mL. Sugar-based gelators readily formed nanoscale lamellar structures to form a coherent network at very low concentrations (1–3% wt./v), which congeal a wide range of lipophilic oils. The efficiency was computed in terms of mechanical, thermal and structural properties and found to be a function of type and concentration of structuring agents. Select examples of biocatalytically enabled molecular gelators and their oleogels will be discussed and are not only be sustainable, but tailored by structure to function as needed.

**Effect of Sunflower Wax on Physical Properties of Oleogels Made with Beeswax and Candelilla Wax.** Jill Winkler-Moser, USDA, ARS, NCAUR, USA; Hong-Sik Hwang, USDA ARS NCAUR, USA; Frederick Felker, USDA ARS NCAUR, USA; Jeffrey Byars, USDA ARS NCAUR, USA; Alejandro Marangoni, University of Guelph, Canada

In order to tailor and optimize the physical properties of oleogels for various food applications, more information is needed to understand how different gelators interact. Building on previous findings that some ratios of beeswax (BW) and candelilla (CLW) interact synergistically to produce firmer oleogels with lower melting points than oleogels made with pure waxes, and that certain ratios of sunflower wax (SFW) and candelilla wax also synergistically formed firmer gels, we studied the effect of ternary wax mixtures containing low levels (10 % of total wax) of sunflower wax with mixtures of BW and CLW in oleogels that had a total of 5% wax. The small amount of sunflower wax increased oleogel firmness at

4°C and 22°C. While the ratio of BW to CLW influenced the solid fat content (SFC) at temperatures between 30°C to 50°C, the addition of SFW had no significant effect on the melting properties. These results will be discussed in relation to observations of the crystal microstructure and SFC during crystallization.

**Engineering Lipid Structure with Enzymatic Glycerolysis.** Reed Nicholson, University of Guelph, Canada

The functionality of an oil is commonly improved by increasing the saturated fat content. We have shown that enzymatic glycerolysis represents a means of directly structuring liquid oils into solid fats without altering the fatty acid composition of the system. In this research, reactions were performed under various conditions using non-specific Candida antarctica lipase B as well as 1,3-specific lipase from Rhizomucor miehei as catalysts. Candida antarctica lipase B performed markedly better than the 1,3-specific lipase at structuring edible oils. Within several oils, glycerolysis resulted in the production of a high-temperature crystallization peak characteristic of a monopalmitin-diolein complex, as demonstrated by differential scanning calorimetry. Pulsed nuclear magnetic resonance demonstrated that optimized reactions increased the solid fat content (SFC) at 5°C from 8% to 29% and 34%, for cottonseed and tigernut oil, respectively. At 20°C, 21% and 8% solids remained in the respective oils. Altering the glycerol concentration present during the reaction changed the composition of partial glyceride species in the glycerolysis product. This resulted in different rates of crystallization, producing different crystal morphologies and fractal dimension values in the reaction products. The oil binding properties of the systems were directly related to the fractal dimension of the crystals. Additionally, both cottonseed and tigernut oil produced margarines demonstrating plastic flow behaviour similar to commercial margarines when subjected to large deformation mechanical testing. This research demonstrates that enzymatic glycerolysis can be used to enhance the functionality of edible oils for use in food applications.

**Impact of Minor Oil Components on Wax Oleogel Properties Such as Critical Gelling Concentration, Crystal Size and Distribution, Firmness and Transition Temperatures.** Maria Scharfe, Technical University Berlin, Germany; Eckhard Floeter, TU Berlin, Germany

Previously, the role of solvent properties, particularly polar oil components (PC), on oleogel properties was discussed for sitosterol/oryzanol. The aforementioned gelling system features a self-assembling fibrillar network. The question arises whether the results are transferable to oleogels from crystalline waxes. Hence, sunflower (SFX), bees (BWX), rice bran (RBX), and candelilla wax (CLX) was utilized to gel edible oils (canola, sunflower) with different levels of PC (purified, natural, deteriorated). The crystal structure was visualized using light microscopy, and image analysis was applied to determine their size and distribution. Physical properties such as transition temperatures and enthalpies, firmness, storage modulus, and break-up behavior were determined by DSC, material testing, and rheology, respectively. The data was linked to solvent properties such as viscosity, POV, free fatty acidy, and polarity. Moreover, a rheological approach to accurately determine the critical gelling concentration (CGC) was developed.

CGC decreased similarly with increasing PC in both oils and was practically invariable with triglyceride composition (purified oil). CGC depended on the wax type and consequently on wax composition and crystal structure. The share of the main wax constituents (wax ester, hydrocarbons, FFA, and FAL), their carbon chain lengths, and homogeneity influence CGC and oleogels firmness. While firmness increased with PC in BWX and CLX oleogels, results were inconsistent for RBX and SFX. DSC data suggests that the solubility of waxes is lower in oils without PC. In contrast to the decline in sol-gel transition temperature with increasing PC reported for the sitosterol/oryzanol system, no such effect could be observed for wax oleogels.

The results indicate that the interactions of PC with the scaffolding elements are different in crystalline and self-assembling oleogel networks. Knowledge about the effect of solvent composition on oleogel properties is vital for thriving food product applications and enables reliable and comparable research.

**Self-assembly of Symmetrical and Asymmetrical Alkyl Esters in Vegetable Oil.** Jorge Toro-Vazquez, UASLP-FCQ, Mexico; Gilda Avendaño-Vásquez, Instituto Tecnológico Superior de Tierra Blanca, Mexico; Anaid De la Peña-Gil, Universidad Autónoma de San Luis Potosí, Mexico; Maria Charó-Alvarado, Universidad Autónoma de San Luis Potosí, Mexico; Miriam Charó-Alonso, Universidad Autónoma de San Luis Potosí, Mexico

Saturated alkyl esters play an important role in determining the functional properties of the vegetable waxes used in the formulations of edible oleogels. We studied the relationship between the thermo-mechanical properties and crystal microstructure developed by saturated symmetrical (SE: 14:14, 16:16, 18:18, 20:20, and 22:22) and asymmetrical (AE: 18:14, 18:16, 18:20, 18:22) esters in the neat state and in oleogels. Additionally, we evaluated the effect of 1-stearoyl-glycerol (MSG; 0.5% and 1%) in the development of SE and AE oleogels. The X-ray and microscopy analysis in the neat state and in oleogels showed that SE self-assembled developing plate-like crystals, while AE developed acicular-like crystals. The AE oleogels had higher elasticity (G′) than the SE oleogels. In both types

of oleogels as the ester carbon number increased the oleogels' G′ decreased and crystal size increased. The addition of just 0.5% MSG, particularly in the AE oleogels, limited the decrease in G′ as the ester carbon number increased, mainly because MSG decreased crystal size. The calorimetry results suggested that during cooling the MSG and the alkyl esters developed a co-crystal. Part of the MSG did not interact with the ester molecules and crystallized independently acting as active fillers of the microstructure. The overall effect was that in comparison with the alkyl ester oleogels the alkyl ester oleogels with 0.5% and 1% MSG had higher G′ with frequency independent rheological behavior. This rheological behavior was particularly evident with the AE oleogels. Therefore, ester composition and molecular structure (i.e., symmetry or asymmetry) greatly influence its molecular self-assembly and subsequently the oleogels' thermo-mechanical properties. Studies using molecular mechanics modeling are underway to establish the mechanism for AE and SE self-assembly with and without MSG. The overall goal is to understand and control the crystallization of vegetable waxes for the development of functional edible oleogels.

**Systematic Study of Waxes and Their Hydrolyzates.** Till Wettlaufer, Technical University Berlin, Germany; Eckhard Flöter, Technical University Berlin, Germany
The soft solid character of lipid phases is crucial for a wide range of product applications. Since triacylglycerides composed of predominantly unsaturated fatty acids cannot deliver the necessary structure usually hardstock fats, high in saturated fatty acids, are used. Alternatively, the so-called oleogellation/organogellation uses non-triglyceride structuring agents to immobilize the liquid oil in three-dimensional lattice. This also results in a structured lipid phase with a semi-solid character. Among various structuring agents, natural waxes appear to be most promising, since they structure liquid oils at low inclusion levels (0,3 - 4 wt%) and are ready available. Since waxes are molecularly rather ill-defined the substantial amount of research contributions on wax oleogels is difficult to combine in a comprehensive manner. It appears hence necessary to map functionality against molecular composition because waxes contain next to various wax esters, free fatty alcohols and fatty acids and other component in sometimes significant quantities. In this contribution the effect of the variation of the ration between wax esters and fatty acid/fatty alcohol is discussed. Due to the approach of mixing waxes with their respective hydrolyzates the overall composition with respect to fatty acid and fatty alcohol moieties remains constant while the molecular composition is systematically varied. These various gels were studied in terms of their macroscopic properties, rheology and firmness, their thermal behavior (DSC), and microstructure (BFM, cSEM).

## Fundamentals of Fat Crystallization III—From Liquid State to Structure

*Chairs Alejandro Marangoni, University of Guelph, Canada; Eckhard Flöter, Technical University Berlin, Germany*

**Dynamics of Complex Fluids: Understanding Aggregation Rates in Food Systems.** David Pink, St Francis Xavier University, Canada; Iris Joye University of Guelph, Canada; Wei Cao, University of Guelph, Canada; Fernanda Peyronel, University of Guelph, Canada; Erzsebet Szabo, St. Francis Xavier University, Canada; Shahjahan Razul, St. Francis Xavier University, Canada; Gurpreet Matharoo, St. Francis Xavier University, Canada
Understanding and predicting how the structures of food systems change with time is important in designing food products. Physical and chemical interactions between different ingredients can lead to time-dependent aggregation on various spatial scales. The intent of this talk is to describe molecular-scale models to study the process of thiol detection and disulfide bond formation in aqueous low-concentration cysteine or glutathione solutions (Lauwers et al, 2016; Cao et al., submitted). Using Ellman's procedure, experiments showed that, in pure MilliQ water, or with added mono- or di-valent salts, the time, tc, to eliminate essentially all thiol groups was finite. However, in what seemed to be a plausible random-diffusion process, the model used to describe the time evolution of the number of thiols remaining at time t, predicted that tc is infinite. It was clear that a complete understanding of the rate of formation of disulfide bonds from thiols in an aqueous solution was missing. To model the process requires equations which describe the mathematics of the rate of disulfide bond formation and which one can use to predict new data. One technique is to use the Smoluchowski equations while another is to utilize molecular dynamics and computer simulation. In this talk, the experimental data of Lauwers et al. (2016) and Cao et al. (submitted) will be briefly described followed by an outline of the model and the application of the Smoluchowski equations, together with a description of the molecular dynamics used. It will be shown that random diffusion and pairwise molecular binding is insufficient to understand the observation of finite tc. An outline of the electrostatic interactions will be given, their effects shown and predictions made, and experimental data exhibited as to how the system evolves as mono- and di-valent salt is incorporated.
Work supported by NSERC and Compute Canada

**Effect of Sucrose Ester S-170 on Physical Properties and Polymorphic Behavior of Cupuassu Fat and Its Fractions.** Maria Lidia Herrera, University of Buenos Aires, Argentina; Ana Carolina Rodriguez

Negrette, University of Buenos Aires, Argentina; Maria Jose Rodriguez Batiller, University of Buenos Aires, Argentina; Virginia Borroni, University of Buenos Aires, Argentina; Victor Alonso Garcia Londoño, University of Buenos Aires, Argentina; Roberto Jorge Candal, University of San Martin, Argentina

Fat from cupuassu beans is extensively used in the production of candies and confectionery in the northern and northeastern regions of Brazil, where the Theobroma species was originally found and is considered as an excellent raw material for utilization in the food industry. Besides, cupuassu fat is considered a good candidate to partially substitute cocoa butter in chocolate. Polymorphism of natural fats is very important for the food industry since it is related to macroscopic properties of final products, such as texture, colour or appearance. Physical chemical properties of cupuassu fat were modified by dry fractionation. The effect of the sucrose ester S-170 on crystallization kinetics and polymorphic behaviour of cupuassu fat (CF) and its stearin fractions obtained by dry fractionation at 29 (S-29), 26 (S-26), and 24 (S-24) °C was studied by polarized light microscopy and small and wide-angle X-ray scattering. In situ experiments were performed in real time using synchrotron radiation as the light source. Sucrose ester S-170 shortened induction times of crystallization, acting as a seed. In addition to the kinetics effects, it modified polymorphic behaviour. Depending on processing conditions used (under thermal cycles or isothermal storage), it promoted crystallization in a specific polymorphic form: β'2 (CF and its stearins) or β2 (S-29). Crystallization in a stable and unique β'-form makes CF and its stearins a good alternative to trans-fats. With the addition of sucrose ester S-170, S-29's increased tendency to crystallize in β2-form extends its applications in chocolate and coatings.

**Free Fatty Acids Presence, Concentration and Their Impact on Cocoa Butter Quality, Crystallization and Chocolate Production: A Systematic Approach Under Static and Dynamic Conditions.** Raul Petrut, Olam Cocoa BV, Netherlands

Nature, presence and concentration of the minor components are key elements in determining the quality and properties of fats and their applications. In the present study, the impact of free fatty acids (FFAs) on cocoa butter (CCB) and chocolate quality, crystallization and production is investigated, employing and comparing a variety of both, industrial QC (i.e., Shukoff, Jensen and BCI) and research methodologies (TD-NMR, DSC, Rheometry). The FFAs concentration was carefully controlled to cover a 0.05 – 2% range, deemed relevant for the world-wide available CCBs. While, the crystallization of CCB is negatively impacted by the presence and concentration level of FFAs under static conditions, it is observed that shearing plays an important role during CCB crystallization and can overcome the FFAs negative impact for the investigated concentrations. In addition, only under shearing conditions the CCB was observed to develop $\beta_V$ crystals, whereas via static crystallization $\beta_{IV}$ was obtained, for the same crystallization temperature and time conditions. Moreover, it is shown that for the FFAs to have a significant ($p < 0.05$) impact on the crystallization and quality of CCB, the FFA increment needs to be >=0.8%. The FFAs presence and concentration are shown to have a more noticeable effect during the polymorphic transformation stage, rather than on crystallization onset, where for some samples FFAs is observed to act as nucleation templates. In chocolate, the FFAs were observed to also impact the texture and handling, in addition to crystallization. The viscosity of the chocolate decreased with increasing level of FFA, while the chocolate bars hardness increased. The bloom appearance and development were not affected by the FFA presence and concentration. These findings also emphasize the need for more suitable and robust approaches towards assessing the crystallization and quality of CCB as a material and as an ingredient in edible applications.

**Palm Oil by Dry Fractionation: Impact of Trisaturated Triacylglycerol Content on the Process Efficiency.** Veronique Gibon, PhD in Chemistry, Desmet Ballestra Group SA; Belgium; Sabine Danthine, University of Liège, Belgium; Gilles Cremer, Gemboux Agro-Bio Tech, Belgium; Christophe Blecker, Gemboux Agro-Bio Tech, Belgium

Palm oil is the most fractionated oil worldwide and is used in many food formulations. Its potential for multistep dry fractionation allows to generate liquid and solid fractions, all having peculiar physicochemical properties. Crystallization cycle times and operation yields are important parameters since they directly impact the process cost. In this context, the study focused on pilot scale production of palm olein IV 56; palm olein is a commodity fat obtained in one single step from palm oil. The aim was to investigate the influence of the trisaturated triacylglycerol content (StStSt) on the process efficiency. Palm oil was blended with palm stearin in order to modify this content; five concentrations were studied: 7.0%, 7.4%, 7.6%, 8.2% and 9.8% of StStSt. The crystallization was monitored during a period of 240 minutes; the oil temperature was recorded, and the crystallization kinetics were determined by p-NMR (SFC). The crystal shapes were characterized by optical microscopy and the polymorphic forms by powder XRD. Two concentrations were shown to crystallize faster. All the crystallized blends were then filtered at 25°C with a pilot membrane press filter. The percentages of crystallization (SFC) at the filtration temperature, the olein yields, the olein IVs, the olein compositions by HPLC and the olein cloud points by DSC were determined. The olein of the two blends crystallizing faster showed slightly higher yield but also

higher cloud point than expected. These olein contained somewhat more palmitic acid in sn-2 position; these observations were explained by unusual co-crystallization behavior in this composition range.

**Prenucleation Structuring in Liquid Triacylglycerols: Navigating a Non-ideal Landscape.** Gianfranco Mazzanti, Dalhousie University, Canada

The thermodynamic of solid-liquid equilibrium currently available for triacylglycerols (TAG) assume that the liquid mixtures behave ideally. The best kinetic models out there have been adapted to follow these thermodynamics as a target equilibrium.

Findings over the last 10 years have shown that these remarkable tools are, alas, insufficient to predict the nucleation and subsequent growth of TAGs and their multicomponent mixtures.

In this talk we will look at the process of discovery that has led to a new understanding of their liquid structures. These structures are ultimately responsible for the nucleation events and the subsequent growth of the TAG multicomponent crystals.

Unexpected phase splittings, uncanny resistance to form glassy states, persistence of 'memory' in the liquid, have been better documented. Study of shearing flow effects provided better understanding of the interactions between the liquid and crystals under formation.

Finally, careful observation of x-ray and neutron scattering data, along with molecular interaction modelling, have narrowed the focus to the importance of the tristar copolymer nature of the TAG molecules.

The combination of the awareness of their local polarity imbalance with their spatial anisotropy, has brought out a picture of the liquid structural landscape that we are just starting to grasp.

The local clustering of the molecules and its dependence on temperature is the most important characteristic of this landscape.

The quantification of its properties (number of molecules, sizes, etc.) are being measured and continue to sharpen our ability to map the local features that ultimately define what happens when we cool a liquid TAG.

An overview of the future of this research is synthesized in a number of questions and possible experimental and theoretical avenues that we shall pursue.

## General Edible Applications Technology I

*Chairs Kaustuv Bhattacharya, University of Mississippi, USA; Supratim Ghosh, University of Saskatchewan, Canada*

**A Review of the Many Forms of Bloom.** Richard Hartel, University of Wisconsin, Madison, USA; Jiayang Jin, East China University of Science and Technology, China (People's Republic); Liana Rodier, University of Wisconsin-Madison, USA

In foods, bloom is usually associated with an eruption on the surface of chocolate or compound coating. Sugar bloom comes from contact of the fatty surface with water, although fat bloom is much more common. Fat bloom is generally associated with spikes of crystalline fat emanating out of the surface, disrupting the flat shiny exterior to give a matte or dull, hazy surface. Numerous theories have been proposed as to the cause of bloom, but, based on the wide variety of bloom forms, it is likely that there are numerous causes.

Chocolate is not the only example of a material that blooms. In fact, a variety of different forms of bloom have been observed and studied on several materials. From efflorescence on leather-bound books or bars of soap, to outgrowths on fat-infused statues, to bloom on mascara sticks and lip balm, surface eruptions occur on a variety of different objects, although the common theme in all of these is fat recrystallization.

In this review, the many forms of fat bloom are reviewed, both in foods and beyond. The primary aim is to look for commonalities and differences in order to categorize the factors that promote bloom formation, to bring the learnings from studies in other fields to bear on chocolate bloom. Such a broad-looking approach may advance our understanding of the mechanisms of bloom formation on chocolates, leading to potential solutions.

**Collapse of Cavitation Cluster Improves Crystallisation Kinetics of Edible Lipid During Sonication.** Jack Youngs, University of Southampton, United Kingdom; Peter Birkin, University of Southampton, United Kingdom; Juhee Lee, Utah State University, USA; Silvana Martini, Utah State University, USA; Tadd Truscott, Utah State University, USA

High-intensity ultrasound (HIU) can play a significant role within the processing of many new food materials, particularly edible lipids, with potential to tailor their physical properties for specific food applications. Although HIU has been shown to enhance the lipid crystallisation kinetics and in turn the physicochemical characteristics of the resultant crystal network, the exact mechanism of its action remains unclear. The nature of the cavitation dynamics present during the HIU treatment could be central to understanding these effects within lipid systems. The crystallisation behaviour of an edible lipid was explored for a set of specific ultrasonic conditions, each characterised by the presence of a different bubble cluster next to the piston-like emitter ultrasonic source employed. This comprised four single cavitation clusters and a unique bifurcated streamer (BiS) event. Reduced induction times were reported in the presence of HIU compared to untreated

control samples, however values were only statistically different between each of the cavitation regimes at low supercooling values. Notably, crystallisation in the presence of the BiS event displayed the greatest energy efficiency and the highest crystal growth rates, with smaller lipid crystals overall compared to higher ultrasonic powers. Samples showed increased values of melting enthalpy and elasticity after 60 minutes when crystallised with HIU, but these changes were most significant at the highest power level. In addition, evidence of attenuation within the acoustic emission was recorded by hydrophone over the 10 second HIU treatment. This attenuation effect was greatest for the highest power level (75 W) and is likely to be associated with the generation of a large gas bubble population within lipid media. Overall, these results indicate that the lipid crystallisation process is sensitive to the dynamics of the cluster and that the action of the BiS event is significant for the energy efficiency of this processing technique.

**SAXS/DSC Characterization of Columbian Cocoa Butter: Influence of Composition on Structure.** Daniel Kalnin, ISTOM, France

Lipids are self-assembling molecules, responsible for compartment formation in living cells. Besides real crystals and bilayers, they also form mesophases thanks to their aptitude to modulate interface curvature. Therefore, lipid-based structures such as solid lipid nanoparticles, liposomes, cubosomes, and other hybrids are interesting for cosmetic pharmaceutical and food applications. This study uses DSC/SWAXS for the monitoring crystalization and phase transitions of coca butter of three different Columbian origins. The crystallization behavior of cocoa butter is monitored using a microcalix calorimeter in a lab setup. Structural changes are monitored with two independent detectors at SAXS and WAXS simultaneously with the DSC signal. Temperature is decreased from 60°C to –10°C at a rate of 4K/min. In that way phase transitions can be attributed to compositional changes.

**Structuring Liquid Oil into Viscoelastic Gel Using Pea Protein Nanoparticles.** Supratim Ghosh, University of Saskatchewan, Canada; Koen Dewettinck, University of Ghent, Belgium; Chi Diem Doan, University of Saskatchewan, Canada

We have recently developed pea protein nanoparticles (PPN) using solvent desolvation method which was used to stabilize Pickering oil-in-water (O/W) emulsions. The objective of the present work was to explore the possibility of PPN in the formation and stabilization of emulsion-templated oleogels. The Pickering emulsions were prepared by mixing oil (50 wt%) and aqueous phase containing PPN (5–10 mg/ml) at different pH values using a high-pressure homogenizer. All emulsions, except the one prepared with 5 mg/ml PPN at pH 3, displayed high stability and strong elastic behavior. Gelation in PPN emulsion was attributed to aggregation of Pickering oil droplets and network of excess PPN holding the droplets in the aqueous phase. At 5 mg/ml PPN, a weak particle network was formed, and the emulsion gel strength was more dependent on droplet aggregation. In contrast, higher particle concentrations thickened the interfacial protein layers and strengthened the continuous phase particle network leading to a strong gel. Freeze-drying was better than oven-drying in creating stronger oleogels with improved rheology and lower oil loss. The oleogels stabilized by 7.5 and 10 mg/ml PPN at pH 7 and 9 revealed about ten-times stronger gel strength and higher stability against alternating shear and gravitational forces than other samples due to smaller droplet sizes and stronger network. In comparison with emulsion-templated oleogels stabilized by conventional molecular proteins, PPN oleogels demonstrated better recovery of gel strength at different shear rates and a higher oil binding capacity. These findings provide important insights into alternative non-trans and less-saturated fat systems for future food applications.

**Study of the Crystallization Behavior of Cocoa Butter: The Influence of Regional Origin in Colombia: Tumaco – Huila – Santander.** Daniel Kalnin, ISTOM, France; Mathilde Bigot, ISTOM, France; Anaëlle Campaignolle, ISTOM, France; Victorine Charpentier, ISTOM, France; Justine Freulard, ISTOM, France; Arthur Messager, ISTOM, France

Theobroma Cacao L., the cocoa tree is a tropical cauliflorous tree that produces pods containing cocoa beans. These beans undergo various stages of processing, resulting in a finished product: chocolate. Cocoa butter constitutes about 55% of the dry weight of cocoa beans is composed of triglycerides. The proportions of their fatty acids vary according to the variety of cocoa, due to climatic, geographical, and genetic factors. This leads to variations in physical properties, such as hardness or melting point, which in terms lead to different organoleptic properties.

Those physical properties influence the temperature that allows the crystallization of cocoa butter called tempering. This is one of the most important steps in the manufacture of chocolate, due to the diversity of fatty acids that make up cocoa butter blends. Indeed, these complex mixtures of triacylglycerols have different melting points. The fatty acid profile observed in cocoa butter is thus the main factor that can influence its organoleptic and rheological properties, as well as its processing qualities during the transformation stages.

We, therefore, studied chocolate from three different regions of Columbia. These premises allow the cocoa trees to be grown under similar climatic and soil conditions and agronomic practices. It is important to notice that the traceability of different kinds of cocoa butter remains difficult. In our case, Luker Chocolate provides chocolate.

Studies show that cocoa butter extracted from hybrid cultivars of Theobroma cacao L"" exhibit relatively constant melting characteristics. Based on genetic diversity but regional homogeneity, we investigated how this regional difference influences the organoleptic properties after the crystallization of cocoa butter.
Thereafter, we demonstrate a difference in phase behavior. We afterward observe a noticeable contrast in organoleptic characteristics upon flavor release, depending on the regional aviation of growth conditions of cocoa butter.

**Thermodynamic and Kinetic Studies on Palm Stearin and Fully Hydrogenated Soybean Oil Binary Systems.** Kiki Chan, University of Toronto, Canada; Fletcher Han, University of Toronto, USA; Yu-Ling Cheng, University of Toronto, Canada; Levente Diosady, University of Toronto, Canada
Palm stearin is a common ingredient in food and soy stearin (full hydrogenated soybean oil) is a neutral edible fat that has applications in the coating of food containers, eating utensils, and food products. In many applications, palm stearin and soy stearin will likely come in contact; however, the interactions between palm stearin and soy stearin have yet to be studied. The application examined in this study is the fortification of bouillon cubes (or chicken cubes). During fortified bouillon cubes manufacturing, molten palm stearin is sprayed onto a mixture that contains micronutrient premix particles that are coated by soy stearin. This study aimed to establish possible interactions between palm stearin and soy stearin by examining their equilibrium and kinetic behaviors in their binary mixtures. To characterize the system's equilibrium behavior, a phase diagram with varying palm stearin and soy stearin compositions was constructed and the kinetic behavior of the binary system was studied using differential scanning calorimetry. Two kinetics parameters were examined in this study: the rate of dissolution of solid soy stearin into molten palm stearin, and the rate of crystallization of binary mixtures. Equilibrium results showed that palm stearin and soy stearin formed an isomorphous mixture. This study provides insight for AOCS members in the food industry and pharmaceutical industry, who may be able to draw on these results for product development.

## General Edible Applications Technology II

*Chairs Supratim Ghosh, University of Saskatchewan, Canada; Kaustuv Bhattacharya, University of Mississippi, USA*

**Fortification of Plant-based Milk with Calcium May Reduce Vitamin D Bioaccessibility: An in Vitro Digestion Study.** Hualu Zhou, University of Massachusetts Amherst, USA
Plant-based milks are increasingly being adopted by consumers as alternatives to bovine milk due to their perceived environmental and ethical benefits. However, many plant-based milks lack key micronutrients that are normally obtained from bovine milk, such as calcium and vitamin D. The purpose of this study was therefore to fortify a common plant-based milk (almond milk) with these two micronutrients and then use a standardized gastrointestinal tract model (INFOGEST) to examine the impact of product formulation on their bioaccessibility. The impact of the incorporation of different forms (CaCl2 versus CaCO3) and concentrations (0, 1, or 2 g per 240 mL serving) of calcium on the physicochemical properties, lipid digestibility, and vitamin D bioaccessibility was examined. The addition of soluble calcium (CaCl2) promoted aggregation of the particles in the almond milk, which was due to the ability of the calcium ions to reduce the negative surface potential and decrease the electrostatic repulsion between the particles. Conversely, the addition of colloidal calcium (CaCO3) did not promote aggregation because there were fewer free calcium ions present. The addition of high levels of calcium (soluble or insoluble) reduced the bioaccessibility of vitamin D, which was attributed to the formation of insoluble calcium soaps in the small intestine. The bioaccessibility of the calcium increased with increasing calcium concentration and was higher for CaCO3 than CaCl2. These findings are useful for the development of nutritionally fortified plant-based milks with improved physicochemical and bioaccessibility properties.

**Impact of Food Matrix Effects on Lipid Digestion and β-carotene Bioaccessibility.** Yunbing Tan, University of Massachusetts, USA; David McClements, University of Massachusetts Amherst, USA
Food, nutrition, and pharmaceutical scientists are carrying out research to elucidate the factors impacting the bioavailability of bioactive agents, such as nutraceuticals, vitamins, minerals, and drugs. Nevertheless, the impact of food matrix effects on the gastrointestinal fate of bioactive substances is often ignored. Bioactive substances are often dispersed in complex food matrices whose composition and structure can change considerably. In this study, the impact of food matrix effects on the digestion and bioaccessibility of β-carotene-enriched model emulsion systems was therefore characterized. In particular, the influence of oil concentration, droplet size, and emulsifier type was examined using a standardized simulated gastrointestinal tract model (INFOGEST). The rate and extent of lipid digestion increased with decreasing droplet size, which was attributed to the increase in lipid surface area exposed to lipase. The bioaccessibility of the β-carotene also increased as the lipid droplet size decreased, being 82.5% for 0.1 μm droplets and 15.0% for 10 μm droplets. Carotenoid bioaccessibility also

depended on emulsifier type, decreasing in the following order: tween 20 > quillaja saponin > gum arabic > sodium caseinate > lyso-lecithin. The observed differences in β-carotene bioaccessibility were attributed to differences in lipid digestion rates, carotenoid release, and carotenoid micellization. These results have important implications for developing carotenoid-fortified functional foods and beverages with improved efficacy.

**Impact of High Intensity Ultrasound on Physiochemical Properties and Oxidative Stability of Enzymatically Modified Menhaden Oil with Caprylic Acid And/or Stearic Acid.** Juhee Lee, Utah State University, USA; Sarah Willett, University of Georgia, USA; Kerry USA; Casimir Akoh, University of Georgia, USA; Silvana Martini, Utah State University, USA

The purpose of this research was to determine the effect of high intensity ultrasound (HIU) on physical properties and oxidative stability of structured lipids. Caprylic acid (C) and stearic acid (S) were incorporated into menhaden oil using Lipozyme® 435 (L) to prepare the following samples: 1) LC 20 (menhaden oil with 20% of caprylic acid), 2) LC 30 (menhaden oil with 30% C), 3) LS 20 (menhaden oil with 20% S), 4) LS 30 (menhaden oil with 30% S), and 5) Blend C (menhaden oil with C and S). Samples were crystallized for 90 min and HIU was applied at 6 min. Physical properties and oxidative stability were evaluated in sonicated and non-sonicated samples. All sonicated samples had statistically higher G′ (LS 20: 20,846 ± 1,083 Pa; LS 30: 61,570 ± 4,085 Pa; LC 20: 603 ± 34 Pa; LC 30: 220 ± 38 Pa; and Blend C: 1,542 ± 115 Pa, $p < 0.05$) than non-sonicated samples. Sonicated LS 30, LC 30, and Blend C had a higher melting enthalpy (8.2 ± 0.4, 2.9 ± 0.1, and 4.5 ± 0.1 J/g, respectively, $P < 0.05$) than the ones obtained without HIU (6.4 ± 0.2, 0.7 ± 0.1, and 3.1 ± 0.2 J/g, respectively, $P > 0.05$), while sonicated LS 20, and LC 20 were not statistically different than the non-sonicated samples (LS 20: 4.6 ± 0.1, 4.1 ± 0.1 J/g; and LC 20: 0.9 ± 0.1,1.3 ± 0.1 J/g; respectively, $P > 0.05$). No significant difference between sonicated and non-sonicated samples was observed in peroxide values (1.2 ± 0.1 meq/kg, $p > 0.05$) and in oil stability index (6.3 ± 0.2 h, $p > 0.05$). These results showed that HIU was effective at changing G′ and melting enthalpy, without affecting the oxidation of the samples.

**The Rheological Properties of Oleocolloid and Hydro-oleocollid Networks.** Clifford Park, Ohio State University, USA; Osvaldo Campanella, The Ohio State University, USA; Farnaz Maleky, The Ohio State University, USA; David McClements, University of Massachusetts Amherst, USA; Hualu Zhou, University of Massachusetts Amherst, USA; Yunbing Tan, University of Massachusetts, USA

Novel gel systems of oleocolloid (OC) and hydro-oleocolloid (HOC) made of whey protein [2.5, 5, or 7.5% w/w] and oleogel were structured and the effects of protein and lipid chemistry on the viscoelastic properties of developed systems were quantified. Small and large deformation tests indicated that OC exhibited a greater gel strength when formulated with higher protein concentrations. However, increasing protein content in HOC resulted in less solid-like properties. Creep-recovery tests indicated that while OCs exhibited a greater gel strength compared to HOCs, the latter was more flexible with a higher % recovery up to 10%. The viscoelastic properties of the networks were further characterized using a model based on fractional calculus that resulted in the following equation J(t) = = (λ1 H(t) λ2 H where α, λ1, and λ2 are mechanical properties of the gel. The parameters α, λ1, and λ2 were obtained from the experimental creep-recovery data. The model showed that all three parameters displayed an inverse relationship with protein concentration for OC. Specifically, α, (a numerical value of elasticity), λ1, and λ2, (the inverse of elastic modulus during creep and recovery, respectively) of OC decreased with increasing protein content. In contrast, α values of HOC were significantly higher compared to OCs and relatively independent of protein content. However, both λ1 and λ2 of HOC linearly correlated with protein content. The observed differences in the viscoelastic properties between OC and HOC matrices indicate the different colloidal interactions mediated by the oleogelation process and the liquid medium type (oil vs. emulsion).

## Implication of Lipids Structuring in Food Application—Part 1

*Chairs Nuria Acevedo, Iowa State University, USA; Brad Forrest, Danisco Australia Pty Ltd, Australia*

**Novel Cocoa Butter Equivalent from Microalgal Butters.** Saeed Ghazani, University of Guelph, Canada; Alejandro Marangoni, University of Guelph, Canada

An acetone solvent fractionation procedure was used to obtain two algal butter fractions, an algal stearin containing high amounts of 1,3-stearoyl-2-oleoyl-glycerol (SOS), and an algal mid-fraction containing high amounts of1,3-dipalmitoyl-2-oleoyl-glycerol (POP), and 1-palmitoyl-2-oleoyl, 3-stearoyl-glycerol (POS). Algal stearin and algal mid-fraction were blended (1:9 w/w) to yield a potential cocoa butter equivalent (CBE). The amount of POP, POS, and SOS,in algal CBE was 15.8%, 32.0%, and 24.6%, respectively, compared to 15.4%, 38.8%, and 27.7% in Cocoa butter (CB). However, a higher amount of POO and SOO in the algal CBEcaused a lower solid fat content at all temperatures compared to CB. This study demonstrates the potential

for producing a new algal CBE using solvent fractionation and blending techniques that can be used in chocolate and other confectionery products

**Textural and Rheological Properties of Sonocrystallized Edible Fats.** Silvana Martini, Utah State University, USA

The objective of this presentation is to summarize recent research related to the use of high-intensity ultrasound (HIU) to improve textural and rheological properties of various edible fats. Results obtained for an interesterified soybean oil (IESBO) with low content of saturated fatty acids, an all-purpose shortening (APS), a palm kernel oil-based fat (PK), oleogels, and butter will be presented.

Sonication of IESBO resulted in a significant increase ($p < 0.05$) in hardness (from 1.5N to 3.4N) and elasticity (10kPa to 30kPa) and these increased values were maintained over storage for 6 months at room temperature. The sonicated sample also showed a a 46–65% reduction in of oil loss depending on the crystallization temperature. Similar increases in hardness and elasticity were obtained for APS.

Sonocrystallization of a PK also resulted in a significantly ($p < 0.05$) harder material (3.4N) compared to the non-sonicated sample (1.4N) that resulted in a reduction of oil loss from 13.2% to 0.3%.

In addition, HIU improved the hardness of a candelilla wax/monoacylglycerol/hardfat oleogel and this increase in hardness resulted in a more stable and harder emulsion gel (from 0.1N to 0.2N) formulated with 25% water. When HIU was used in a monoglyceride (MG)-based oleogel, harder materials were obtained for oleogels formulated with 3% MG (0.09N vs. 0.05N for sonicated and non-sonicated samples, respectively), 4.5% MG (0.26N vs. 0.14N for sonicated and non-sonicated samples, respectively, and 6% MG (0.45N vs. 0.29N for sonicated and non-sonicated samples, respectively). Similar trends were obtained for G′ values of these samples.

HIU was also used in cream to generate harder butters. Butters formulated with sonicated cream were harder (57.9N) compared to their non-sonicated counterparts (34.6N; $p < 0.05$).

Overall, this presentation will show that HIU can be used as an additional processing technology to improve the physical properties of fats.

## Implication of Lipids Structuring in Food Application—Part 2

*Chair Nuria Acevedo, Iowa State University, USA; Brad Forrest, Danisco Australia Pty Ltd, Australia*

**Margarines from Wax-based Oleogels: Improved Properties with Mixtures of Candelilla Wax and Beeswax.** Hong-Sik Hwang, USDA ARS NCAUR, USA; Jill Winkler-Moser, USDA, ARS, NCAUR, USA

Partially hydrogenated vegetable oils containing trans fats have been widely used in textured food products such as margarines. However, due to recent regulations banning trans fats, food companies have replaced partially hydrogenated vegetable oils with highly saturated fats such as palm oil and fully hydrogenated vegetable oils, which have negative health effects including elevation of LDL cholesterol levels and dysfunction of liver lipid metabolism. Oleogels have high potential as alternatives to highly saturated fats in margarines. Among many oleogels studied, natural wax-based oleogels have unique advantages including relatively low melting point, high oil binding capacity, high gel strength, and low cost. However, wax oleogel-based margarines typically have higher melting points than conventional margarine, which can result in an undesirable waxy mouthcoating when consumed. In this study, margarine samples were prepared from oleogels with binary mixtures of candelilla wax (CDW) and beeswax (BW) in soybean oil and their physical properties were examined. The DSC study showed eutectic melting properties of margarines with mixtures of CDW and BW indicating that the melting point of wax-based margarines can be lowered by mixing two waxes. Solid fat content (SFC) at different temperatures also showed the eutectic properties of margarines with the binary wax system, showing the lowest SFC values with margarines with 50 or 75% BW. In addition to the advantage of lower melting points, some margarines made with mixtures of CDW and BW had higher firmness than those with pure wax. Strong gel strength is an important property of oleogels, which can provide the desired firmness with a small amount of oleogelator resulting in a minimal effect on sensory properties of the final product. Therefore, this study showed that properties of oleogel-based margarines such as firmness and melting properties could be improved by mixing two waxes.

**Oleogelation of Canola Oil Using Canola Protein Isolate-stabilized Concentrated Oil-in-water Emulsions.** YanRan Tang, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

Canola protein isolate (CPI)-stabilized oleogel was developed using an emulsion-templated approach, and its application in cake baking was investigated. First, 50 wt% oil-in-water emulsions stabilized with 1 and 4 wt % CPI were developed using high-pressure homogenization. Subsequently, the emulsions were vacuum-dried at 60 °C and sheared to form oleogels. The emulsions were also heat-treated (90 °C for 30 min) prior to drying to improve interfacial strength, oleogel stability and gel strength. The 4% CPI heated emulsion (HE)-templated oleogel exhibited the lowest oil loss (4.3%)

and highest G′ (~75000 Pa) and firmness (47.5 ± 16.7 N). Oil droplets surrounded by canola proteins were observed in the confocal micrograph of 4% CPI emulsion oleogels while the droplets in 1% CPI emulsion oleogels were disrupted due to high-speed shearing. The 4% CPI emulsion oleogels were chosen as shortening replacer in a cake baking study. The shortening cake batter showed lower specific gravity and the highest viscosity and G′, while those of oleogels were not significantly different than canola oil batter. Confocal micrograph of shortening cake batters showed small air bubbles entrapped in the continuous fat phase while larger air bubbles and oil droplets were observed in the oleogel and canola oil cake batters. The cakes made from HE oleogel was significantly darker in colour compared to the cakes made with unheated emulsions (UE) oleogel and shortening. The HE oleogel cakes showed lower hardness, higher cohesiveness and springiness than those of the shortening cake. The different attributes of the cake with different fat phases could have arisen from the ability of the fat phase stabilizing the air bubbles and their interaction with the cake matrix. Overall, the findings contribute insight into the utilization of canola proteins in developing oleogels for cake baking.

**Relationship Between Oil Binding Capacity and Physical Properties of Interesterified Soybean Oil.** Melissa Marsh, Utah State University, USA; Silvana Martini, Utah State University, USA

The objective of this study was to identify the physical properties of an interesterified soybean oil (EIESOY) containing 45% of saturated fatty acids (SFA) that correlate with high oil binding capacity (OBC). In this study, two EIESOY samples were analyzed; a 100% EIESOY sample and a 50% EIESOY sample diluted with 50% soybean oil. Both EIESOY samples were crystallized using fast (10°C/min) and slow (0.1°C/min) cooling rates as well as with and without high-intensity ultrasound (HIU, 20kHz) to generate a wide range of physical properties and oil binding capacity. The 100% EIESOY samples were crystallized at 38.5°C and the 50% EIESOY samples were crystallized at 27°C. For the fast-cooled samples, HIU was applied after 27 and 10 min into the crystallization process for the 100% and 50% EIESOY, respectively. For the slow-cooled samples, HIU was applied at the onset of crystallization. All samples were allowed to crystallize isothermally for 90 min and physical properties such as crystal morphology, hardness, solid fat content (SFC), elasticity, and melting behavior were evaluated. Physical properties were also measured after storage at 22°C and 5°C for 48h including oil loss (OL) and OBC. Results show that for the 100% EIESOY sample, OBC measured at 5°C was negatively correlated (R=–0.981, p-value=0.019) with the second melting peak obtained after 48h storage at 5°C and positively correlated (R=0.992, p-value=0.008) with the enthalpy of the second melting peak obtained after 48h storage at 5°C. For the 50% EIESOY sample, SFC at 90 min was negatively correlated with OL at 22°C (R=–0.983, p-value=0.017 and OL at 5°C (R=–0.987, p-value=0.013). Overall, this research shows that OBC can be increased by formulating fats that melt at lower temperatures and that have high enthalpy values. Additionally, for a sample with lower content of SFA, lower OL can be achieved by increasing the SFC.

**Structuring of Vegetable Oil by γ-oryzanol Crystallization.** Khakhanang Wijarnprecha, Ryerson University, Canada; Pravit Santiwattana, Thai Edible Oil Co., Thailand; sopark sonwai, Silpakorn University, Thailand; Dérick Rousseau, Ryerson University, Toronto, Canada

γ-oryzanol extracted from rice bran oil consists of a series of compounds that result from the esterification of ferulic acid and a triterpene alcohol or sterol. Many reports have shown that γ-oryzanol readily gels vegetable oil when mixed with a number of sterols, and that alone, it does not gel oil. Here, we demonstrate that in the presence of combined cooling and mixing, γ-oryzanol can structure vegetable oil at concentrations as low as 7 wt%. At a temperature below its crystallization onset, quiescent processing does not result in oil structuring, given the formation of γ-oryzanol crystal spherulites upwards of 100 μm in diameter. At both 'low' (50 rpm) and 'high' (700 rpm) mixing rates, network formation occurs, with the higher rate resulting in platelet crystals a few microns in length and the equilibrium solids content being reached within the preparation timeframe of 1 h. For a given mixing and cooling regime, higher γ-oryzanol contents increase the resistance to melting, elastic modulus and hardness. Overall, this study reveals that γ-oryzanol can singlehandedly structure vegetable oil if appropriate combined mixing and cooling are used.

**Study of the Crystallization Behavior of Cocoa Butter: The Influence of Geographic Origin by Countries: Ivory Coast – Colombia – Perou.** Daniel Kalnin, ISTOM, France; Lina Morel, ISTOM, France; Marion Tailliez ISTOM, France

Cocoa butter (CB) is an essential ingredient of chocolate and has a direct influence on the consumer relevant characteristics of this globally consumed product. The crystallization properties of CB influence the tempering process, a necessary step in the production of quality chocolate. The composition and hence the hardness of CB can vary considerably depending on the geographic origin, particularly with the environmental temperature at the time of maturation of the beans. Additionally, aroma compounds as they exist in fine cacao (e.g., Colombia) can influence crystallization behaviour. Besides, CB composition can influence the delivery of those aroma compounds. Mastery of

crystallization behaviour is key for the process of tempering, which is necessary for high-quality chocolate. We studied the influence of the environmental temperature at the time of maturation of the beans on the crystallization of CB. We compared CB from three distinct locations in Ivory Coast, Peru, and Colombia with commercial-grade CB. Differential scanning calorimetry and X-ray diffraction were used to monitor and compare the crystallization behaviours of known origins obtained after different thermal treatments. The results show a notable physicochemical difference between the different CBs. Notably, they differ in their final melting points but we can follow different melting behaviour indicating differences in composition. We were able to compare composition data with physicochemical properties. The findings encourage further experimentation with the knowledge of the CB agronomic practices, which are perfectly identifiable, to understand precisely the influence of these agronomic parameters on the crystallization of CB

**Applications of New Short Path Stripping Technology in Edible Oil Processing.** Ernesto Hernandez, Advanced Lipid Consultants, USA

Thin film evaporation and short path distillation address challenges associated with processing heat-sensitive oils that require short residence times, high heat and mass transfer coefficients and shorter contact times. This processes normally operate at lower temperatures and higher vacuums in the processing heat-sensitive oils like omega 3 oils and now are also widely used in the removal of processing and environmental contaminants from oils like palm, soybean and other marine oils.

Artisan's new short path stripping (SPS) technology, with internal multistage and condensing capabilities, takes advantage of both principles, namely short path distillation and thin film evaporation. The SPS offers several advantages over commercial molecular distillation and deodorizer systems including applications in the removal of contaminants, physical refining and recovery and concentration of omega 3 ethyl esters.

The new SPS system was tested on a variety of vegetable oils and temperature sensitive products, including palm, soybean and omega 3 oils for the removal of processing contaminants in soybean and palm oils (MCDPs and GEs), removal of free fatty acids for physical refining and concentration of ethyl ester of omega 3 fatty acids. The oils were analyzed for fatty acid composition, MCPDs, GEs, free fatty acids, mono and diglycerides, peroxide value, anisidine value, color and flavor. The omega 3 ethyl esters were analyzed for fatty acid composition, free fatty acids, peroxide value and anisidine value. The resulting oils met commercial quality specifications for refined oils including reduction of free fatty acids from 3.87% to < 0.1% in palm oil. Regulatory specs for MCPDs and GEs were also successfully met and the SPS system effectively concentrated omega 3 ethyl esters to over 50 % EPA and DHA.

## Manipulating Fat for Plant Based Product Development

*Chairs Karel Hrncirik, Upfield, Netherlands; Farnaz Maleky, The Ohio State University, USA*

**Chickpea Aquafaba as an Emulsifier in Food-oil Emulsion.** Yue He, University of Saskatchewan Canada; Rana Mustafa, Government of Saskatchewan, Canada; Timothy Tse, University of Saskatchewan, Canada; Venkatesh Meda, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada

Aquafaba is defined as a viscous liquid produced during boiling or pressure-cooking of chickpea seed or other legumes. This by-product solution has been gaining popularity as an egg replacement in many food products (e.g., mayonnaise, ice cream and baked goods) due to its foaming and emulsion properties. As different seeds and legumes may produce varying amounts of aquafaba, our research objectives were to select a chickpea cultivar that produces superior aquafaba and to standardize aquafaba cooking conditions. Five chickpea cultivars, produced by the Crop Development Center at the University of Saskatchewan, were selected (CDC Leader, CDC Orion, CDC Consul, CDC Luna, and Amit). Chickpea seed cooking conditions were investigated and optimized to produce aquafaba demonstrating excellent emulsion capacity and stability. Our results demonstrated that aquafaba produced from CDC Leader had the best emulsion properties when soaked at 4 °C for 16 h and cooked for 30 min at 116 °C, 75 kPa in a pressure cooker. Furthermore, drying aquafaba via lyophilization and spray-drying produced powder that also maintained the functionality of aquafaba after rehydration. However, spray-drying removed most water (95 g water/100 g aquafaba sample), in the shortest time frame (0.29 h) when compared to other drying methods (e.g., freeze-drying, oven-drying, rotary evaporation-drying, and vacuum-drying). The emulsification properties of aquafaba are greatly influenced by the chickpea genotype, as well as the cooking and drying conditions. Therefore, to ensure the consistent quality of aquafaba produced, standardization processes must be developed to address these variables.

**Effect of High-intensity Ultrasound on the Oleogelation and Physical Properties of High Melting Point Monoglycerides and Triglycerides Oleogels.** Thais da Silva, ULiege, Netherlands; Sabine Danthine, University of Liège, Belgium

Oleogels and oleogelation routes have been extensively studied in the past decade; however, the industry has not yet implemented this technique due to price, availability and clean label. The objective of this study was to evaluate the synergism of binary oleogels structured by monoglycerides (MG) and high melting point

triacylglycerols (HF) with and without high-intensity ultrasound (HIU) according to their physical properties. MG:HF (0:6, 1:5, 2:4, 3:3, 4:2, 5:1 and 6:0) oleogels were produced by mixing at 70°C with a stirring of 350rpm for 5 min, followed by a cooling and storage at 20°C for 24h. A 20kHz HIU was applied for 10s, 30s or 10s using 3 pulses (10sON/10sOFF) during the cooling step via macro tip (12.7mm) and 50% amplitude (56W) in the presence of few crystals. Samples were evaluated according to their hardness, oil binding capacity (OBC), microstructure, melting behavior, viscoelasticity and flow behavior. The best physical properties were found in the MG6:HF0 oleogel, with a hardness of 1.2N, elasticity of 5.5kPa, viscosity of 99Pa.s and 99% OBC. These properties were reduced with the decrease of MG in the blend. The sonication did not improve the MG6:HF0, instead it affected its properties negatively. However, sonication showed a positive effect on the blends of MG and HF. The hardness was improved at least 3-fold and OBC around 20%, these effects were already observed using only 10s sonication. Sonocrystallization induced secondary nucleation and changed the crystalline material only in blends containing HF indicating the better effect of the sonocrystallization on oleogels in the presence of high-melting points triacylglycerols.

**Goat Cheeses Elaboration with Increased Content of Conjugated Linoleic Acid and Transvaccenic Acid.** Ignacio Vieitez Osorio, PEDECIBA Quimica-UdelaR, Uruguay; Cecilia Dauber, UdelaR, Uruguay; Tatiana Carreras, UdelaR, Uruguay; Alejandro Britos, UdelaR, USA; Silvana Carro, UdelaR, USA; Cecilia Cajarville, UdelaR, USA; Adriana Gámbaro, UdelaR, USA; Santiago Jorcin, UdelaR, USA; Tomas Lopez, UdelaR, USA

Goat's milk and its dairy products have been commonly related to important nutritional and health benefits. In fact, fat fraction is one of the most important components of goat's milk regarding its value, as lipids are involved in cheese yield, firmness, color and flavor of goat dairy products. The aim of this study was to evaluate the modification in the lipid and sensory profile of cheeses elaborated with goat milk, through diet inclusion of sunflower oil (SFO). The main objective was to elaborate Danbo Type cheeses with increased content of conjugated linoleic acid (CLA) and transvaccenic acid (TVA), and at the same time decrease the proportion of saturated fat, and evaluate these values during the shelf life of the cheeses. The study was conducted with 60 Saanen goats separated into two homogeneous groups and supplemented for 18 days with one of two concentrates: without added oil (control, C) or enriched with sunflower oil (SFO). Cheeses were elaborated using goat milk, collected after desirable changes in its lipid profile were achieved. Physicochemical, microbiological, and fatty acid composition together with the sensory profile were evaluated. In SFO cheeses fat content increased, without affecting protein content, microbiological, sensory profile and consumer acceptability. Also, the content of TVA increased from 2.4 to 4.4% ($p < 0.01$), while CLA presented an increase from 0.8 to 1.3 % ($p < 0.01$). A decrease in the saturated/unsaturated ratio was observed resulting in a decrease in the atherogenic index. Therefore, inclusion of SFO in the diet of goats could be a viable alternative to increase the contribution of fatty acids with beneficial effects for health in cheeses.

**Impact of Network Architecture and Lipid Physical State on the Mechanical Properties and Scaling Behavior of Emulsion-filled Gelatin Gels.** Andrew Gravelle, University of Guelph, Canada; Alejandro Marangoni, University of Guelph, Canada

In many fat-containing foods, the lipid phase exists as discrete droplets embedded in a continuous matrix. The dispersed fat can be viewed as filler particles which directly contribute to the deformation and fracture behavior of the overall product. This work aims to address the effect of network architecture on the mechanical properties of emulsion-filled protein gels as a fat- or oil-filled composite food matrix. Emulsions stabilized with whey protein isolate were incorporated into gelatin gels at varying filler content (0–30 wt%) and gelator concentration (2–8 wt%). Through pH adjustment, filled gels having either homogeneous or heterogeneous network architectures were produced. Heterogeneous gels were characterized by regions of droplet-rich, protein dense structures embedded in the continuous gelatin matrix. Both systems displayed a characteristic reinforcement in the relative elastic modulus (Er) with increasing filler content, which was dependent on droplet and matrix stiffness. The homogeneous gels followed the general behavior predicted by the van der Poel model of particle reinforcement. The observed reinforcement was affected by lipid physical state, but was unexpectedly independent of gelator concentration. The latter was attributed to the availability of positively charged amino acid sidechains responsible for filler/matrix adhesion. Er of the heterogeneous filled gels could not be described by the van der Poel model, but followed a power law scaling behavior with increasing filler content. This response was attributed to an increase in the number of load-bearing structures able to translate stress through the composite network. Such scaling behavior is analogous to that observed in aggregated colloidal gels and fat crystal networks. This work demonstrates that network architecture plays a pivotal role in determining the linear elastic response of fat-filled protein gel networks.

**Polysaccharides-based Aqueous Foams, Cryogels and Oleogels: Characterization, Interrelation, and Formation Mechanism.** Zong Meng, Jiangnan University, China (People's Republic); Qinbo Jiang, Jiangnan

University, China (People's Republic); Yuanfa Liu, Jiangnan University, China (People's Republic)
The application of renewable substances in edible soft materials has been gaining particular concerns, especially the replacement of saturated fats. This work presented a lightweight and highly oil-absorbed cryogel fabricated by two polysaccharides, hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG), as the network matrix via the foam-templated method, and the pathway for preparing oleogels with the cryogel, containing low-saturated fats. The thickening effect of XG dramatically stabilized bubbles, avoiding the bubble collapse during cryogelation, which separated the network structure from waters, greatly heightening the oil absorption capacity of freeze-dried cryogels and enlarging the porous network structure. The overrun value was lowered gradually through raising the HPMC, which further reduced the porosity, total pore volume, and oil absorption capacity of the freeze-dried cryogel from aqueous foam. The increment of HPMC could reinforce the network of cryogels and generate smaller pores that had a stronger capillary force for absorbing oils. Physical properties of oleogels were directly inherited from cryogels to a great extent that viscoelasticity-enhanced oleogels could be prepared by the cryogel possessing a more compact network, meaning the key point of the foam-templated method to make oleogels was the adjustment of cryogels. The complex and disordered pore structure in cryogels could be considered as that pores generally had the uniform size, according to two absorption kinetic models, which accurately fitted experiment data of cryogels. Furthermore, the oleogel was endowed with the structural recovery ability in the presence of XG. The HPMC improved the resistibility of temperatures and the structural recovery for oleogels. These findings might provide a deeper understanding of oleogels fabricated from the foam-template, promoting applications of oleogels as a vital role in replacing trans fats in foods.

**Structuring Bigels as an Alternative to Animal Fat in Coarse Ground Meat Products.** Rodrigo Tarte, Iowa State University, USA; Nicole Kibler, Iowa State University, USA; Nuria Acevedo, Iowa State University, USA
This work entailed the development of edible bigels produced by a combination of a soybean oil-based oleogel and a gelatin hydrogel to replace the animal fat in coarse ground meat products. Four oleogel/hydrogel ratios (80/20, 60/40, 50/50 and 40/60) were prepared. Oleogels contained 90% high oleic soybean oil (HOSO) and 10% w/w rice bran wax (RBW), while hydrogels were formulated with 7% w/w gelatin in water. Cooked smoked sausage controls were formulated to a 28.5% fat target. The prepared bigels were used to replace 100% of the fatty pork trim, which resulted in a total fat replacement of at least 90%. The progress of physicochemical (lipid oxidation, texture, color) and sensory properties of the formulated sausage products were followed for 98 days in sausages stored at 1–2°C. Across all time points, TPA hardness and chewiness of smoked sausages were higher in the pork-fat control samples than in the bigel treatments; whereas no differences were found in resilience and springiness across treatments. On the other hand, the internal color of sausages prepared with bigel was lighter than the controls. Lipid oxidation (TBARS) were higher in bigel treatments than in controls at all time points. Sausages with the 7:3 bigel had higher TBARS values than those with 6:4 bigels, indicating higher levels of lipid oxidation possibly due to higher oil content. Despite the observed differences, all samples remained below commonly accepted sensory threshold values for this type of meat product. Controls have more intense smoked sausage aroma and moisture release than all bigel treatments. All treatments had more intense off-aroma and off-flavor than controls. 6:4 bigel treatments showed more distinct internal and external particle definition, as well as darker color, than controls and 7:3 bigel treatments. The formulated bigels closely reproduced the organoleptic properties of animal fat in smoked sausage.

## Phase Transition and Interfacial Phenomena in Complex Food System—Part 1

*Chairs Filip Van Bockstaele, Ghent University, Belgium; Andrew Gravelle, University of Guelph, Canada*

**Encapsulation of Tannic Acid in Pea Protein Nanoparticles for Improved Oxidative Stability of Flaxseed Oil-in-water Pickering Emulsion.** Supratim Ghosh, University of Saskatchewan, Canada; Andrea García Guzmán, University of Saskatchewan, Canada; Dien Chi Doan, University of Saskatchewan, Canada; Fatemeh Keivaninahr, University of Saskatchewan, Canada
The present work aimed to encapsulate tannic acid (TA) in alcohol de-solvated pea protein nanoparticles (PPN) to stabilize Pickering oil-in-water emulsions for improved protection against lipid oxidation. It was hypothesized that the presence of encapsulated TA at the PPN-coated oil droplet surface would be better to prevent lipid oxidation than when TA would be present in the bulk phases. Pea protein isolate was dissolved at pH 9, and the soluble protein fraction (PPS) was isolated by centrifugation. The de-solvated PPN was prepared by adding two-volumes of ethanol to the PPS solution drop-by-drop while stirring, followed by centrifugation to collect the nanoparticles. For encapsulation, TA was dissolved in ethanol before the de-solvation process. Pickering oil-in-water emulsions (pH7) were prepared by high-pressure homogenizing 5wt% flaxseed oil with PPN dispersions (with or without

encapsulated TA). As a control, PPN emulsions were also prepared with equal TA concentration added to the bulk oil or aqueous phases. TA-PPN emulsions were formed with a lower average droplet size (188 nm) than PPN emulsion without TA (253 nm). PPN emulsions with TA in the bulk phases were extensively aggregated due to the lowering of emulsion pH by free TA in the aqueous phase (TA in oil droplets also leached into the aqueous phase). Peroxide value of flaxseed oil for TA-PPN emulsions stored at 60ºC increased with time for 3-weeks, followed by a significant drop after 4-weeks, far better than the PPN emulsions without TA, but worse than when TA was present in the bulk phases. In contrast, p-anisidine values of TA-PPN emulsions at 60ºC were lowest compared to all other emulsions. These results indicate that highly stable Pickering emulsions stabilized with TA encapsulated in PPN could be a novel way to provide improved long-term oxidative stability of flaxseed oil emulsions.

**Investigation of Stabilization Mechanism of Glycerol Monooleate in Water-in-oil Emulsions Using FTIR and Thermal Analysis.** Maria Romero-Pena, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

The stabilization mechanism of liquid water-in-oil (W/O) emulsion was investigated by analyzing hydrogen (H) bonding between glycerol monooleate (GMO) and aqueous phase hydroxyl group (OH) donating agents ascorbic acid (AA) and low methoxyl pectin (LMP). It was hypothesized that the OH-donor agent forms H-bonds with the GMO OH groups, thereby keeping them at the oil-water interface. The W/O emulsions were prepared with 80% canola (CO) or mineral (MO) oils containing 4% GMO and 20% aqueous phase, including either sodium chloride (S), AA, their mixture, or calcium chloride (Ca), LMP, their mixture using high-pressure homogenization. LMP-based emulsions exhibited the highest stability. All emulsions were analyzed with differential scanning calorimetry (DSC) and mid-IR spectroscopy. The water and GMO melting peaks from DSC were analyzed for temperature shift and enthalpy to show the H-bonding strength. Ice melting peaks exhibited more H-bonding in MO-emulsions prepared with S, AA, and Ca than CO; however, the opposite was true for LMP-emulsions. All GMO melting peaks with S, AA, and LMP demonstrated their significant interaction in CO than MO. More H-bonding were developed among CO, GMO and LMP at the interface. In mid-IR, deuterium oxide was used as the aqueous phase, so GMO intermolecular and intramolecular H-bonding was analyzed without water interference. All intermolecular GMO-H bonding reported higher peak areas in CO than MO, indicating a strong bond among GMO-OH groups with CO. All intermolecular GMO carbonyl H-bonding exhibited significant peak areas in CO than MO, suggesting greater interaction in CO. The GMO a-carbonyl revealed substantial H-bonding with the OH-donor agents than intermolecular GMO-bonding. LMP and AA showed further intermolecular H-bonding with GMO hydroxyl and carbonyl groups. Both analyses demonstrated that liquid W/O emulsions' stability comes from GMO intermolecular interactions with OH-donating agents at the interface.

**Stability of Soy Protein-isoflavone Particles and Pickering Emulsions.** Garinn Pereira, University of Hawaii at Manoa, USA; Kacie Ho, University of Hawaii at Manoa, USA

Colloidal oil-in-water emulsions are commonly found in food and beverage systems. Particularly, Pickering emulsions are stabilized by interfacial solid particles and offer improved stability via irreversible absorption. Polyphenols (plant derived secondary metabolites) are capable of complexing with proteins through structural interactions. Certain polyphenols have shown to influence interfacial properties of proteins, though use of isoflavones has yet to be evaluated. Thus, the objective of this research is to understand the interaction of isoflavones on emulsion stability. Varying concentrations of isoflavones were complexed to soy proteins through pH differentiation and induced heating. Physical characteristics (size, zeta potential, and polydispersity index) of soy-isoflavone particles and emulsion droplets (5% oil fraction) were measured using a Zetasizer Nano ZS. HPLC analysis and the Folin-Ciocalteu method were performed to identify and quantify the primary isoflavones used for complexation. Particles formulated with higher concentrations (5 - 25mg/mL) of isoflavones had significantly smaller particle sizes and greater repulsive energy. Emulsion droplets exhibited similar effects with the addition of 20mg/mL of isoflavones having the most significant change in size and 25mg/mL displaying the strongest repulsive energy. Overall, emulsion droplets sustained size and zeta potential over a 7-day evaluation. Soy-isoflavone particles are a potential alternative to existing emulsifying ingredients in the food industry. Overall, this work demonstrates the use of particles for food grade colloidal dispersions and further expands the knowledge for incorporating phenolics as functional ingredients in emulsion systems.

**Stabilization Mechanism of Water-in-oil Emulsions by Medium- and Long-chain Diacylglycerol: Post-crystallization vs. Pre-crystallization.** Guanghui Li, Jinan University, China (People's Republic); Xuanxuan Lu, Jinan University, China (People's Republic); Chaoying Qiu, Jinan University, China (People's Republic); Yong Wang, Jinan University, China (People's Republic)

Medium- and long-chain diacylglycerol (MLCD) has superior nutritious features and surface activities. To reveal its crystallization features in emulsions, the

influence of crystallization process (pre- or post-crystallization) on the stability of MLCD-based emulsions was examined by pulsed field gradient NMR droplet size analysis, sedimentation experiments, microscopy and differential scanning calorimetry. The flocculation/coalescence rate was reduced with increasing MLCD concentration from 2 wt% up to 8 wt%, among which 5 wt% MLCD is required to provide sufficient crystals for formation of stable emulsion. Compared to the pre-crystallized emulsion, the post-crystallized emulsion showed narrower droplet size distribution (DSD) and less sedimentation during storage. Thermal analysis revealed that the irregular crystallization peak of water droplets formed in pre-crystallized emulsion under low shear speed was correlated with larger droplet size. Microscopy indicated the presence of interfacial crystal shell in post-crystallized emulsion and the formation of large crystals in the continuous phase of the pre-crystallized emulsion. With shear speed increasing to 10,000 rpm, the decrease in d3,3 values and sedimentation was partly attributed to the breakup of spherulite as smaller crystals. Due to the formation of interfacial crystal shell, the post-crystallized emulsion had higher viscoelasticity and was efficiently prevented from flocculation or coalescence. The observations could be the basis for future application of MLCD and provide insights on how the crystallization process influences the physical stability of emulsions.

**Sustainable Particles at the Oil-water Interfaces.** Anwesha Sarkar, University of Leeds, United Kingdom

Dispersions of liquid emulsion droplets or gas bubbles stabilized by colloidal particles (via the Pickering stabilization mechanism) are highly resilient towards coalescence and Ostwald ripening as compared with conventional dispersions stabilized by surfactants or polymers. In the last decade, major advances have occurred in the use of plant-based colloidal particles for the stabilization of oil-in-water [1] and water-in-oil emulsions [2]. In this talk, the characteristics of polysaccharide-based particles, protein-based particles and organic crystals (flavonoids) with respect to their particle size, degree of aggregation, anisotropy, hydrophobicity and electrical charge. Specific effects of processing on particle functionality will be discussed [1]. Particularly, a case study on stabilization of emulsions by pea protein microgels and their ability to provide gastric stability to the emulsions in presence of cellulose nanocrystals will be discussed [3]. Special emphasis will be directed towards the issue of correctly defining the stabilization mechanism to distinguish those cases where the particles are acting as genuine Pickering stabilizers, through direct monolayer adsorption at the liquid–liquid interface, from those cases where the particles are predominantly behaving as 'structuring agents' between droplets without necessarily adsorbing at the interface, for example, in many so-called high internal phase Pickering emulsions. Finally, outlook for future research activity in the field of Pickering emulsions for food applications will be highlighted.

References

[1] Sarkar A, Dickinson E. 2020. Sustainable food-grade Pickering emulsions stabilized by plant-based particles. Current Opinion in Colloid and Interface Science. 49, pp. 69–81.

[2] Zembyla M, Murray BS, Sarkar A. 2020. Water-in-oil emulsions stabilized by surfactants, biopolymers and/or particles: A review. Trends in Food Science & Technology. 104, pp. 49–59.

[2] Zhang S, Holmes M, Ettelaie R, Sarkar A. 2020. Pea protein microgel particles as Pickering stabilisers of oil-in-water emulsions: Responsiveness to pH and ionic strength. Food Hydrocolloids. 102 Article No. 105583.

## Phase Transition and Interfacial Phenomena in Complex Food System—Part 2

*Chairs Andrew Gravelle, University of Guelph, Canada; Filip Van Bockstaele, Ghent University; Belgium; Lulu Dong, South China Agricultural University, China (People's Republic)*

**In Vitro Gastrointestinal Digestibility of Phytosterol Oleogels: Influence of Self-assembled Microstructures on Emulsification Efficiency and Lipase Activity.** Yaqi Lan, South China Agricultural University, China (People's Republic)

The objective of this study was to investigate the influence of a self-assembled microstructure on lipid digestibility in phytosterol (γ-oryzanol and β-sitosterol) oleogels. Different molar ratios of γ-oryzanol and β-sitosterol yielded a variety of crystal morphologies; the resulting gels were tested for their lipid emulsification efficiency, release rate of free fatty acids (FFAs) during lipolysis, and their effect on lipase behavior. Results indicated that oleogels with stronger gel strength were harder to emulsify when compared to oil samples. Phytosterol oleogel with an equal molar ratio of γ-oryzanol and β-sitosterol produced nanoscale tubular crystals and displayed enhanced emulsification efficiency when compared to phytosterol oleo gels of other molar ratios. In oil emulsions, intestinal digestion resulted in more extensive lipid droplet coalescence with increased particle size when compared to oleogel emulsions. The FFAs release rate suggested that the extent of lipid digestion was correlated to the emulsification efficiency, which was influenced by both the microstructure and gel strength of a given oleogel. The interfacial binding of lipase indicated that the amount of lipase adsorption was positively correlated to the interface area created during the emulsification process. Finally, isothermal titration calorimetry results indicated that self-assembled structures within these oleogels physically obstructed the interaction between

lipase and lipid. Ultimately, this led to lower reaction rate during gastrointestinal digestion. Collectively, these results may have important implications in designing oleogel systems with controlled lipid digestibility as well as controlling the bioavailability of delivered lipid-soluble bioactive compounds.

**Interfacial Particles for Controlling Flow Properties and Creating Functional Materials.** Paul Clegg, University of Edinburgh, United Kingdom
Nanoparticles and larger colloids can become trapped at liquid interfaces provided they have appropriate surface chemistry. The particles modify the elastic properties, permeability and interactions of the interfaces. As a consequence, they are important building blocks for designing delivery technologies, modifying flow properties and for creating functional materials. For example, this approach has been used to create unique tricontinuous media, growing droplets and capsules which can be switchable or can give burst release. In this talk, I will show how functionality is a consequence of the mesoscale size of the particles.

**in-vitro Digestion of Water-in-oil Emulsions Stabilized with Fat Crystals.** Jonathan Andrade, Ryerson University, Canada; Vivekkumar Patel, Ryerson University, Canada; Dérick Rousseau, Ryerson University, Toronto, Canada
The purpose of this research was to investigate the effect of emulsifier type and presence of solid fat on the stability and dye release characteristics of water-in-oil (W/O) emulsions subjected to simulated gastrointestinal conditions. Emulsions consisting of a 20 wt% aqueous phase dispersed in canola oil were stabilized in one of four different ways: core-shell stabilization with glycerol monostearate (GMS), network stabilization using polyglycerol polyricinoleate (PGPR) and solid fat added to the continuous phase (PGPR-F), combined core-shell and network stabilization using glycerol monooleate and a continuous phase fat crystal network (GMO-F) and finally, a PGPR-based liquid emulsion with no added fat. The dispersed aqueous phase of all emulsions contained 1 mM methylene blue (MB), which was used as a marker to quantify emulsion breakdown and release of aqueous phase cargo. When subjected to gastric conditions, the PGPR-F emulsion showed the lowest MB release after 60 min (< 3% of initial load) with the other emulsions showing ~ 12% release. In duodenal conditions, the PGPR-F and GMS emulsions showed the lowest MB release after 120 min of exposure (~5%) followed by the PGPR (9.4%) and GMO-F (14.6%) emulsions, respectively. Emulsion photomicrographs taken prior to, and after, contact with simulated gastric and intestinal fluids showed that emulsion microstructure was an important contributor to emulsion stability. Overall, the PGPR-F emulsion was the most stable in both gastric and intestinal fluids. These results have shown that fat phase structuring is key parameter to control W/O emulsion breakdown behaviour in simulated gastrointestinal conditions.

**Rheology and Digestion Behaviour of Mono- and Bilayer Oil-in-water Nanoemulsions.** Kunal Kadiya, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada
Emulsion gels are widely used in different foods where flow behaviour is restricted due to either jamming or the formation of a network of oil droplets. In this work, it was hypothesized that jamming could be induced by decreasing the oil droplet size and increasing the interfacial shell layer thickness. The primary whey protein stabilized nanoemulsion with the lowest droplet size of 200 nm was prepared by high-pressure homogenization. The excess whey protein from the continuous phase was removed by ultracentrifugation which was followed by redispersion and homogenization of the nanoemulsion. In secondary emulsions, pectin with different degree of esterification (DE: 33% and 73%) and concentration (0 and 1wt%) was used to form layer-by-layer electrostatic deposition at pH 3 on positively charged whey protein stabilized oil droplets. The aim was to determine the influence of the interfacial thickness on the gelation and digestion behaviour of resultant nanoemulsion in the presence and absence of excess whey protein in the continuous phase. At 1wt% pectin (DE 33 and 73), nanoemulsions containing excess protein showed bridging flocculation, however, after removal of excess protein, both the emulsions showed the lowest droplet size, where zeta potential reached beyond the charge neutralization with more adsorption of pectin on WPI stabilized primary emulsions. The secondary emulsions showed a significant increase in viscosity and viscoelasticity which can be attributed to an increase in interfacial thickness due to the presence of steric bilayer at the interface and an increase in the droplet charge with a decrease in DE value of pectin. The digestion behaviour was also significantly affected by the deposition of the pectin layer on the WPI stabilized oil droplet. The gelation in emulsion achieved at a much lower oil concentration, using the bilayer approach, can serve as an attractive option to produce low-fat food products with controlled digestion.

**The Effect of Different Emulsifiers on the Physical, Thermal and Microstructural Properties of a Rice Bran Wax—Gelatin Bigel System.** Ariana Saffold, Iowa State University, USA; Nuria Acevedo, Iowa State University, USA
A bigel is a biphasic system that is composed of an organogel and hydrogel combined at a high shear rate. Although bigels offer greater stability than other gelled systems due to its semi-solid internal phase, emulsifiers are often used to further stabilize the system and

prevent separation of phases. The objectives of this study were to determine the role of different emulsifiers in stabilization of a rice bran wax – gelatin bigel system and examine their effect on the physical, thermal and structural properties. Bigels were formulated with three different emulsifiers (Tween 80, Mono-diglycerides, PGPR) at four emulsifier concentrations (0.5, 1, 2, 3% (w/w)). The organogel-to-hydrogel (OG:HG) ratios tested were 60:40, 70:30 and 80:20. Bigels with the same OG:HG ratios prepared without emulsifiers were the experimental controls. The hydrogelator and organogelator concentrations remained as 7% and 10% (w/w), respectively, for all samples. Bigels were analyzed using nuclear magnetic resonance spectroscopy (NMR), differential scanning calorimetry (DSC), fluorescence microscopy, texture analysis and liquid binding capacity studies. HLB value, particle size and concentration affected the ability of the emulsifiers to stabilize the system; only the most hydrophobic emulsifier (PGPR) was able to stabilize the 80:20 OG:HG ratio. Despite that the physical stability of most bigels increased in the presence of emulsifiers, textural hardness generally decreased with increasing emulsifier concentration. Additionally, DSC analysis showed a decrease in the degree of destabilization in bigels with PGPR when compared to other emulsifiers and the controls after two cycles of heating and cooling. The quantity of liquid lost from a leaching study remained under 10% for all samples, with water contributing to the majority of leached internal phase.

## Insect Fat-Food Industry and Science Prospective

*Chair Diliara Iassonova, Cargill Inc, USA*

**Are Consumers Ready to Eat Insect Oils?** Daylan Tzompa Sosa, Ghent University, Belgium
In the last decade insects had emerged as a new source of oils and fats with applications in the feed industry. However, the food industry could also benefit from this novel source of oils and fats but better food applications need to be developed and consumer acceptance should increase. In this presentation we will discuss the chemical and physical characteristics of these lipids and we will compare them to those of conventional edible oils. Moreover, we will discuss the use of insect oils and fats in food applications and the need of product development to adequate these products aiming an increase in consumer acceptance.''

**Fat Boys Are Back! Black Soldier Fly Lipids and Their Value.** Jeff Tomberlin, Texas A&M University, USA
The black soldier fly is used as a means to recycle organic waste and produce insect biomass. This process has proven to be highly effective at preventing wastes from being placed in landfills and potentially polluting the environment while producing products of high value. The digested waste can be used as a fertilizer, while the protein from the insect biomass can be used as feed for select fish species as well as poultry, swine, dogs, and cats depending on location. The fat, which can be up to 60% of the insect biomass, has unique value as well. Such materials have been shown to be a great substrate for producing bioenergy. Other uses include, but are not limited to, diet formulations in animal feeds as well. The purpose of this presentation is to give a general overview of the black soldier fly industry and describe these products of value with an emphasis on the fat associated with black soldier fly larvae. More specifically, how, what, when, and where these fats are produced by black soldier fly will be discussed. Lipid attributes in meat-producing animals that ingest black soldier fly lipids will also be addressed. Furthermore, speculations on methods for enhancing production and quality will be reviewed.
Insect Oils as an Alternative Source for Feed, Food and Industrial Applications. Xiaolan Luo, Cargill, USA
Insect oil is a second-largest component in insect. It recently has attracted increasing attention as a sustainable oil source for feed, food and industrial applications. In the presentation, we will review background of insect industry, discuss the opportunities and challenges of using insect oils as an alternative, and review the current and potential future applications of insect oils in feed, food, fuel, cosmetics, surfactants, and materials.''

## Lipid Oxidation in Frying—Updates and New Perspectives

*Chairs Kaustuv Bhattacharya, IFF, Denmark; Hong-Sik Hwang, USDA ARS NCAUR, USA*

**How to Select the Right Oil for Frying.** Nils Hinrichsen, ADM Hamburg AG, Germany
Statistically 25–36% of North American adults consume foods; usually fried, from fast food restaurants every day. In addition, fried foods pick up potentially up to 30 % of the frying media they are prepared in. Accordingly, the nutritional values and quality of frying fats and oils can have influence on health.
There are different aspects that play a role in the right choice of a frying fat. Of course, oils with a high level of poly unsaturated fatty acids are generally regarded as healthier compared to fully saturated oils, but they are also more susceptible to oxidation especially under the conditions found in deep frying applications. In the past stable frying fats have been produced by partial hydrogenation, but since these contain disproportionally high

levels of trans fatty acids they have been replaced in the more recent years mostly with high oleic liquid oil or palm oil-based products.
Additional stabilization could be achieved by the addition of antioxidants or polydimethylsiloxane. Since most of the antioxidants are volatile, they are rather more effective during the storage of the oils than during the frying itself.
This presentation reviews the pros and cons of different oils and additives used in frying applications.

**In Search of a Unified Theory of Frying Chemistry: Is It Possible?** Karen Schaich, Rutgers University, USA
For decades, arguments have persisted over whether thermal degradation of oils was driven by thermal scissions or accelerated autoxidation. These two theories were derived from data obtained under very different conditions – oxidation after measuring product evolution during long-term frying and thermal scission from rapid short-term heating. Are these mechanisms mutually exclusive or do they work together in series or in parallel? Is the difference even important? The degradation chemistry is very different in fresh oil at the beginning of heating, in commercial "restaurant-type" frying oils heatedly intermittently for many days to weeks, in industrial operations having rapid oil turnovers of a few hours, and in any heated oils immediately after dilution with fresh oils, yet we treat all situations the same when designing stabilization strategies. This paper attempts to integrate shifts in thermal degradation processes from early heating to runaway degradation into a unified theory that can guide adjustments to stabilization strategies under different frying regimens and as frying degradation progresses.

**Lipid-derived Aldehyde Degradation Under Thermal Conditions and Their Scavenging by Phenolics During Food Frying.** Francisco J. Hidalgo, CSIC-Instituto de la Grasa, Spain; Rosario Zamora, CSIC-Instituto de la Grasa, Spain
When frying is carried out, water and other foods components play a role on the lipid oxidation process. Among others, changes in the ratio among lipid oxidation products are observed. In addition, the scavenging of lipid-derived reactive carbonyls by food components is produced. The objective of this paper is both to study the stability of secondary lipid oxidation products upon heating in the presence of water and air, and to discuss the ability of food components to scavenge the formed lipid-derived products. Obtained results show that, in the presence of water and air, 2-alkenals and 2,4-alkadienals are not stable upon heating and the formation of formaldehyde, acetaldehyde, and the aldehydes corresponding to the breakage of the carbon-carbon double bonds is rapidly produced. In addition, some of these compounds are scavenged by the nucleophiles present in the food. Thus, the removal of toxicologically relevant aldehydes occurs during deep-frying when phenol-rich foods are fried.

**Natural Antioxidants for Frying: Barriers to Commercialization and Implementation.** Jill Winkler-Moser, USDA ARS NCAUR, USA; Hong-Sik Hwang, USDA ARS NCAUR, USA
Deep-fat frying is a complicated and dynamic food process. While frying is an ancient process, the techniques, equipment, and ingredients used in fried foods are continually changing and expanding. There is great interest in developing natural antioxidants and other new technologies to improve oil quality during frying, as well as to enhance the nutritional and sensory qualities and extend the shelf-life of fried foods. However, very few natural antioxidants have been successfully developed and marketed for these purposes for a variety of technical, economic, and regulatory reasons. In this presentation, we'll review our research on antioxidants used for frying, best practices for evaluation of antioxidants during frying studies, and some of the technical barriers and knowledge gaps to implementation and commercialization of natural antioxidants for frying oil and shelf-life improvement.

**Study of the Acidic Compounds Derived from Triacylglycerol That Contribute to Acid Value Increment.** Masayoshi Sakaino, J-Oil Mills, Inc., Japan; Takashi Sano, J-Oil Mills, Inc., USA; Shunji Kato, Tohoku University, Japan; Junya Ito, Tohoku University, USA; Jun Imagi, J-Oil Mills, Inc., USA; Kiyotaka Nakagawa, Tohoku University, USA
Objectives: Even acid value (AV) has been recognized as a method to measure free fatty acids (FFA) derived from triglyceride hydrolysis, however, several studies still reported an increment of AV in frying oil under the no-water addition condition. We hypothesized that the AV increment might happen due to the formation of other acidic substances during the thermal oxidation of oil, besides the FFA that generated via triglyceride hydrolysis, which has also been likely calculated as AV. In this study, we identified and quantified the acidic compounds formed in heated oil.
Methods: The pure canola oil was heated under water and no-water addition. FFA and the acidic compounds generated from the heated oil were treated with 9-anthryldiazomethane reagent, which will react selectively with carboxyl moieties, and then will be identified by liquid chromatography coupled to mass spectrometry (LC-MS). Also, the acidic compounds formed during oil heating process were analyzed using gas chromatography coupled to mass spectrometry.
Results and Discussion: The AV that corresponded to FFA concentration was only 10% of the overall titrated AV on the heated oil under no-water condition. During the thermal oxidation, dioleoyl-(9-carboxy-nonanoyl)-triacylglycerol (OOAz-TG) and oleoyl-linoleoyl-

(9-carboxy-nonanoyl)-triacylglycerol (OLAz-TG) were detected as the unique acidic compounds by LC-MS analysis. These compounds were considered to also be calculated as AV from the carboxylic moieties. We also confirmed this finding while cooking French fries that FFA and the acidic compounds were also increased, and the contribution of the acidic compounds to AV increment was higher than FFA. This study has successfully demonstrated for the first time about the identification of acidic compounds which preferentially formed during the cooking process with canola oil under low-water availability condition. Hence, AV should have to be considered as an indicator that evaluates not only triglyceride hydrolysis by water but also the thermal decomposition of triglyceride.

## Emerging Edible Applications of Plant Proteins

*Chairs Navam Hettiarachchy, University of Arkansas-Fayetteville, USA; Long Zou (Joe), Bunge North America, USA; Serpil Metin, Cargill, USA*

**Enzymatic Clean Label Processing for Plant-based Meat Improvement of Binding Property of Textured Vegetable Protein.** Akiko Takahashi, Amano Enzyme Inc., Japan; Takakiyo Obara, Amano Enzyme Inc., Japan; Masamichi Okada, Amano Enzyme Inc., Japan; Shotaro Yamaguchi, Amano Enzyme Inc., Japan

Recently, the market value of plant-based meat has been explosively increasing, for the increase of interests in sustainable society. Changes in dietary preference are also accelerating the trend. Variable products of plant-based meat have been developed and most of them are needed to use food additive binder because of the lower binding property of textured vegetable protein (TVP). We examined to improve the binding property of TVP by the use of enzymes.

Pea or soy TVPs were treated by 0.1–0.3 % (w/w-TVP) proteases at 40–65 °C for 1 hour at the soaking step. After draining, treated TVPs were kneaded with/without other ingredients, such as pea protein powder, olive oil, vegetable block and salt. After molding, putties were baked with olive or sunflower oil. Various shape types, flake, ground, block, of TVP were used.

We found that protease treatment improved the binding properties of all the shape types of both pea and soy TVPs. Compared to no protease treatment, protease-treated TVPs were remarkably easy to be molded as a putty and kept the shape even after baking. The degree of improvement depended on shape types; flake type was very high, block type was high, ground type was middle. Figure 1 shows an example indicating the improvement effects of protease treatment.

These results indicate that proteases treatment of TVP makes it easy to be molded as a putty without food additive binder. This study opens the possibility of clean label plant-based meat analogue by the use of enzyme.

**Heat-induced Gelation and Stabilization of Pea and Faba Bean Protein Concentrate-stabilized Oil-in-water Emulsions.** Fatemeh Keivaninahr, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

Pea protein (PPC) and faba bean protein concentrates (FBPC) (4–10%) were used to prepare 40% canola oil-in-water emulsions using a high-speed blender. The effect of protein concentration, salt addition (1–3%), pH (pH 3, 4.5 and 6.5), and heating (90ºC for 30 min) on the droplet size, charge, microstructure, and rheological properties of the emulsions were compared. Emulsion stability was also investigated after freeze-thaw cycle. The protein suspensions (without oil) formed aggregates and provided larger average particle size (22.3 μm) and smaller zeta potential (–9.3 mV) compared to the emulsions droplets (10.30 μm and –33.4 mV). Addition of salt did not change the droplet size, which, however, increased by decreasing the pH from 6.5 (10.3 μm in average) to 4.5 and 3 (14–18 μm). Storage moduli of all emulsions were higher than the loss moduli which showed the formation of gel. Emulsions viscosity and gel strength increased with protein concentration and decrease in pH, but were not affected by salt addition. Heating the emulsions led to a significant increase in viscosity, storage moduli, and the formation of strong self-supporting gels due to protein denaturation-induced droplet and protein network formation. Freeze-thaw cycle, however, promoted emulsion destabilization with higher free oil separation at lower protein concentration (>90% oil release at 6% protein compared to 57% oil release at 10% protein). Interestingly, heating the emulsions significantly improved freeze-thaw stability with almost no oil separation for all samples which can be due to the increase in interfacial strength due to heat-induced protein denaturation and subsequent aggregation at the interface. Improved stability and gelation behavior of heated emulsions would help to promote their application as fat replacer in a variety of foods, including processed meats.

**Processing Technology and Emerging Applications of Oat Proteins.** Keshun Liu, U.S. Department of Agriculture, USA

Among grains, oat is unique in the presence of avenanthramides, a group of phenolic alkaloids having strong antioxidant effects. Oat also contains mixed linkage (1–4, 1–3) beta-D-glucan, a soluble fiber having health benefits. In recent years, oat has emerged as a promising source of edible proteins, due to its high protein content and high protein quality. Various methods have been developed to enrich or extract oat protein

and other valuable components. Dry methods involve milling, sieving and/or air classification while wet methods entail solvent extraction, precipitation and solid-liquid separation. Dry fractionation is possible due to uneven distribution of protein and other components within oat grain. Both dry and wet methods have been commercialized to produce oat protein concentrates. For fully capturing oat values, at USDA, we have developed an improved method that can fractionate oats into several fractions simultaneously, each enriched with protein, beta-glucan, starch, or other carbohydrates, respectively. With respect to edible applications, the use of oat protein concentrate in liquid and semi-solid foods is still limited due to its low solubility and poor functionality. However, oat protein and related products are widely used in breakfast cereals, bars, and bakery products. They are particularly priced in the gluten-free market, because most coeliac individuals can tolerate oat protein. Most recently, meat analogs using oat protein products have been developed. It is anticipated that, with increasing demand for plant proteins and advancing technologies, oat protein ingredients with better functionality and wider uses will be developed.

**Structural and Functional Properties of moringa Oleifera Seed Proteins.** Rotimi Aluko, University of Manitoba, Canada; Taiwo Aderinola, Federal University of Technology, Akure, Nigeria, Nigeria

Moringa oleifera is an underutilized vegetable that is now widely cultivated in many tropical regions of the world but there is scant information on potential role of the seed proteins as food ingredients. The aim of this work was to determine the structural and functional properties of M. oleifera seed protein fractions in comparison to the whole protein isolate. The two major protein fractions were prepared from a 0.5 M NaCl extract of defatted Moringa flour, which was dialyzed against water to give water-soluble albumin (ALB) and water-insoluble globulin (GLO). The protein isolate (ISO) was obtained after alkaline extraction followed by acid precipitation at pH 5.0. The ISO, GLO and ALB were then analyzed for structural properties such as polypeptide composition, surface hydrophobicity (So), intrinsic fluorescence and conformation (secondary and tertiary structures). Functional properties were evaluated using in vitro protein digestibility, bulk density, oil and water holding capacity, gelation, emulsification and foaming. Results showed that GLO had more $\beta$-sheet conformation while the $\alpha$-helix content varied depending on the pH. Gel electrophoresis revealed that the major protein in the three protein products had a molecular weight of ~20 kDa, which consisted of disulfide bonds. ALB had the highest surface hydrophobicity (946.6), which indicate a more open structure when compared to GLO (7.8) and ISO (50.4). In contrast, the GLO had the highest protein digestibility (89%) in comparison to ALB (81%) and GLO (83%). ALB and ISO had least gelation concentration of 0.8%, which is significantly ($p < 0.05$) lower than the 2.2% for the GLO. The ALB and ISO also had significantly ($p < 0.05$) higher emulsifying properties than the GLO. At 10, 20 and 40 mg/ mL protein concentrations, foaming capacity was comparable in all the samples.

**Technical Opportunities Associated with Producing Plant-based Meat Protein.** Tasha Hermes, Cargill Inc, USA; Serpil Metin, Cargill, USA

It is not new news that the plant-based meat protein category is growing. The category has seen strong growth and billions of dollars of investment. According to a consumer research study conducted by the International Food Information Council (IFFIC), the top reason consumers chose plant-based, outside of novelty is because they are trying to eat less meat and they thought it would taste good. This perception for the plant-based category has not always existed, what is different now? There are a few factors but the most important is attributed to advancement in sensory performance. The taste, texture, appearance and even store placement of newer plant-based meat proteins have reduced the gap between the incumbent meat offering and the plant-based solution. Although this progress is positive, a meat eater will tell you it isn't there yet and that they are making a sacrifice when choosing the plant-based meat option. What are those sacrifices? Where are the research opportunities? Based upon industry experience developing and manufacturing these products, customer and customer research it has been shown that the gaps differ by product type. Plant-based patties mimicking beef vary from plant-based patties mimicking chicken. Opportunities linked to advancing the category overall include, but are not limited to texture delivery through technologies, and new protein sources, flavor, texture and appearance solutions for plant-based fat that mimics the animal incumbent. The opportunity is large for this category and all of us working to unlock technical hurdles associated with taste, texture and appearance of plant-based meat will elevate the entire category.

## Edible Applications Technology Poster Session

*Poster Chair Supratim Ghosh, University of Saskatchewan, Canada*

**Cannabidiol oil quality control with near-infrared spectroscopy.** Adam Hopkins, Metrohm, USA; Elena Hagemann, Metrohm, USA; Andreas Agerer, Metrohm, USA

Cannabidiol is a popular natural remedy used in many pharmaceutical, food, and cosmetic products. Better known as CBD, it is one of over 100 chemical

compounds found in the marijuana plant. Unlike tetrahydrocannabinol (THC), CBD is not psychoactive. This characteristic makes CBD an appealing option for those who are looking for relief from pain and other symptoms without the mind-altering effects of marijuana. CBD oil is made by extracting CBD from the cannabis plant, then diluting it with a carrier oil.
In this talk, we demonstrate the ability of near-infrared (NIR) spectroscopy to determine the CBD content in several different carrier oils, including hemp seed oil, fish oil, and medium chain triglyceride oil. The results point to the potential of building a universal calibration for CBD oil products.

**Carnauba wax oleogel as potential replacer of saturated fat in ice cream formulations.** Roberta Silva, North Carolina A&T State University, USA; Rafaela Airoldi, Sao Paulo University, USA; Silvana Martini, Utah State University, USA; Zacchary Cooper, Utah State University, USA; Juliana Ract, Sao Paulo University, USA; Heather Colleran, North Carolina A&T State University, USA; Thais da Silva, ULiege, Netherlands; Salam Ibrahim, North Carolina A&T State University, USA
Oleogel systems are a potential alternative to replace milk lipid fraction on ice cream formulations. The objective of this study was to develop a wax-oleogel (soybean oil – SO, peanut oil – PO, and carnauba wax – CW) to be used as a fat replacer in ice cream formulations (50% and 100% of fat replacement) The SO and PO oleogels were structured with CW (6, 8 and 10%) and were characterized by oil binding capacity (OBC), visual evaluation, thermal properties (DSC), and microstructure by polarized light microscopy (PLM). All oleogels resulted in a firm and stable gel throughout the 60 days of the analysis period, regardless of the concentration (6, 8 and 10%) and temperatures studied (5 and 25 °C). The OBC of oleogels at 8 and 10 % addition were significantly higher ($p < 0.05$) than the oleogels with 6% of CW. However, the oleogel formed with 6% of CW (SO or PO) showed more than 84% of oil retention after 60 days, indicating that the 6% of CW as an organogelator was sufficient to develop a network that could hold the liquid oil into a gel-like structure. The melting peak temperatures (Tm) increased as the wax content was higher, adding 10% of CW the Tm was 81.5±0.2°C (PO) and 79.3±1.5°C (SO). Larger crystals (diameter) were observed with 10% of CW addition (2.15 ±0.16mm SO and 2.08 ± 0.22mm PO). The SO oleogel with 6% of CW was chosen to be applied in the ice-cream formulation. The ice cream preparations were analyzed by overrun, melting rate, fat composition and sensorial acceptance. The oleogel fat replacement (50 and 100%) reduced the melting rate of ice creams; however, the substitution negatively affected the ice cream's overrun. The sensory evaluation showed positive acceptance for overall liking, texture, appearance and flavor.

**Characterization of main physicochemical parameters of milk obtained from goats supplemented with sunflower and fish oils.** Dario Cabezas, UNQ - CONICET, Argentina; Claudia Dome, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina; Yasmín Carrión Sad, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina; Gerardo A. Gagliostro, Instituto Nacional de Tecnología Agropecuaria (INTA-EEA Balcarce), Argentina; Liliana Antonacci, Instituto Nacional de Tecnología Agropecuaria (INTA-EEA Balcarce), Argentina; Sandra Medici, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata and CONICET, Argentina; María Pereyra, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina; Lorena A. Mignino, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina
Milk is a complete food containing bioactive fatty acids (FA) like the cis-9, trans-11C18:2 (CLA) isomer of the linoleic acid and its precursor, the trans-11C18:1 or vaccenic acid (VA) that showed promising cardioprotective and antitumoral properties. Supplementing goats with polyunsaturated fatty acids (PUFA) is an effective way to increase CLA and VA and reduce saturated fat contents but the effects on milk physicochemical parameters and protein profile are poorly explored. Moreover, Milk fat (MF) and protein content determine the technological properties of the obtained cheese. This work focused on the effects of supplementary PUFA feeding on milk FA profile, casein (CN) composition and other parameters of technological interest of milk obtained from PUFA supplemented goats (PUFA-M) compared to unsupplemented control goats (Control-M). To obtain the PUFA-M, goats were supplemented with sunflower and fish oils at a 5:1 ratio. Proximal analysis, Milk FA composition, fat globule (FG), and CN fractions were analyzed together with density, pH, and acidity. MF content (g.L-1) resulted higher ($P < 0.05$) in PUFA-M (59.67) compared to Control-M (43.82). The same results were observed for lactose (46.85 versus 43.82 g.L-1) and total solids (144.41 versus 134.22 g.L-1). Feeding supplementary PUFA significantly reduced (42.5%) the atherogenic index of milk ($P < 0.05$), with an increase in VA and CLA. FG size was not affected, averaging 5 μm diameter. Although milk protein and total CN were not affected, the PUFA-M showed a higher ($P < 0.05$) percentage of α-1CN fraction, potentially improving coagulation properties coupled to a higher pH (6.79 versus 6.63, $P < 0.05$) and lower acidity (0.137 versus 0.182 mL NaOH/mL milk, $P < 0.05$). Results obtained confirmed that feeding supplementary PUFA is a useful tool to increase bioactive FA content in goat milk reducing its atherogenic index. Also, these milk physicochemical characteristics could improve the cheese-making process and quality.

**Consistency testing of pure triglyceride calorimetric data.** Julia Seilert, Technical University of Berlin, Germany; Eckhard Flöter, Technical University Berlin, Germany

Reliable data are essential to understand how materials and compositions behave. In fat-based products, understanding the solid-liquid phase behavior of triglycerides demands data on the enthalpy of fusion, entropy of fusion, and melting temperature of a large quantity and high quality. Determining these data experimentally (e.g., by DSC) is a function of sample purity and the experimental conditions often influenced by kinetics. This contribution presents a newly formulated criterion to test the consistency of calorimetric data of pure components. Therefore, the well-known linear correlation of the named properties to the carbon number is reviewed for triglycerides and related homologous alkyl-series, i.e., n-alkanes and fatty acids. Furthermore, a direct correlation of the enthalpy and entropy of fusion is discussed. This appears to be natural for n-alkanes but was also found for fatty acids and triglycerides. A linear fit revealed discrepancies between saturated and unsaturated molecules but independence from polymorphic forms and chain length differences in mixed-acid triglycerides. Moreover, the slopes of the linear fits for data on n-alkanes, saturated fatty acids, and saturated triglycerides were found to be of the same magnitude and in the vicinity of the melting temperature of polyethylene. By using the consistency criterion not only the data consistency of experimental data can be reviewed but also for predictions obtained from any model predicting these properties. Further, the criterion allows predicting currently unavailable experimental data on the enthalpy of fusion or melting temperature if at least one is present.

**Destabilization of particle-stabilized water-in-oil emulsions using non-ionic surfactants.** Malek El-Aooiti, Ryerson University; Canada; Auke de Vries, Ryerson University, Canada; Dérick Rousseau, Ryerson University, Toronto, Canada

Particle-stabilized water-in-oil (W/O) emulsions are commonly sought for applications that demand long-term stability against coalescence. However, their remarkable stability may pose problems for applications that require controlled breakdown. Here, we investigated the controlled destabilization of a W/O emulsion stabilized by a high-melting, crystalline surfactant, glycerol monostearate (GMS), which was used to kinetically stabilize model emulsions. Emulsion destabilization was promoted by addition of sorbitan monooleate (SMO), a non-ionic, liquid-state surfactant, which altered the wetting behaviour of the GMS crystals, leading to their desorption from the oil-water interface. The adhesion tension of the interfacially-bound GMS crystals was used as a tuning parameter to quantify the changes in GMS crystal wettability following SMO addition. Changes in droplet morphology resulting from SMO addition were characterized using light microscopy and cryo-scanning electron microscopy (cryo-SEM). Further efforts showed that, in general, surfactants with a higher affinity for the oil-water interface were more effective at destabilizing the GMS-stabilized W/O emulsions. This was attributed to their ability to disrupt the interfacial ordering of GMS crystals at the interface, thereby promoting droplet coalescence. Emulsion destabilization was also contingent on the ability of the surfactants to modify the wettability of interfacially bound GMS crystals. These results further contribute to the knowledge base on particle-stabilized emulsions for controlled release applications.

**Development of nanostructured lipid carriers composed of simple and interesterified blends.** Kamila Godoi, UNICAMP, Brazil; Mayanny Silva, UNICAMP, Brazil; Ana Paula Ribeiro, UNICAMP, Brazil

This study aimed to develop nanostructured lipid carriers nanoparticles (NLCs), using edible fats, oils, and emulsifiers. NLCs were developed by high-pressure homogenization (2 cycles/700bar) with 10% of lipid phase and 90% (w/w) of aqueous phase. Four systems were developed composed of simple and chemically interesterified mixtures of fully hydroganated microalgae oil (FHMO) and soybean oil (SO) in different proportions (w:w): 90:10, 80:20, 70:30 and 60:40 as lipid phase. For the all systems, the hydrolyzed soy lecithin was used (2%) The NLCs were evaluated according to melting behavior, recrystallization index (RI), solid fat content (SFC), polymorphism, droplet size, polydispersity, zeta potential, and stability with crystallization at 5°C for 24 hours and storage at 25°C for 60 days. The FHMO proportion added in the NLCs has a direct influence on physical parameters while the treatment of the lipid phase (simple mixture or randomized) influences the stability of the system. The NLCs composed of lower quantity of FHMO and randomized lipid fraction showed lower thermal resistance, SFC, and size of droplets with greater homogeneity than simple lipid fraction being more resistant to destabilization in the function of time. Both NLCs lipid fraction showed lower RI and $\beta$ polymorphic habit. These physical characteristics and the increase of stability over the storage time could promote the magnification of these NLCs containing randomized mixtures of FHMO and SO as a system for delivery of bioactive compounds, principally lipid food enrichment, such as spreads, margarine or until beverages.

**Distilled Monoglycerides as Low SAFA Fat Structurant for Filling Fat in Sandwich Biscuits.** Maslia Manja Zaman, Sime Darby Plantation Research, Malaysia; Norliza Saparin, Sime Darby Plantation Research, Malaysia; Ahmadilfitri Md Noor, Sime Darby Plantation Research Sdn Bhd, Malaysia

Filling fat in sandwich biscuits are usually made from saturated fat (SAFA) which is important for texture and organoleptic attributes. However, healthier version food products with lower SAFA content are high in demand nowadays. In this study, a reduced SAFA filling fat was prepared from blend of palm stearin IV 28 (PSTIV28), palm mid fraction IV 45 (PMF45) and soybean (SBO) in three different texturized fat formulations namely 10:50:45 (w/w/w), 20:50:30 (w/w/w), 30:20:50 (w/w/w) via oleogelation technique using distilled monoglyceride as structurant. The physicochemical properties of the texturized fat blends and sensory evaluation were evaluated and compared with shortening made with 100% palm oil. Fatty acid composition results revealed that formulation 30:20:50 (w/w/w) had the lowest SAFA (42.6%) whilst shortening made from 100% palm oil had the highest SAFA (49.5%). Slip melting point and crystallization rate were the highest for formulation 30:20:50 (w/w/w) followed by 20:50:30 (w/w/w), 10:50:45 (w/w/w) and 100% palm oil. Formulation that has the highest crystallization rate which is 30:20:50 (w/w/w) was applied as filling fat in sandwich biscuits and compared with filling fat made with 100% palm oil. 7-points hedonic sensory evaluation was conducted by 84 untrained panelists, filling fat made from formulation 30:20:50 (w/w/w) had the highest hedonic scores in terms of taste and melting profile as compared to filling fat made with 100% palm oil. This finding suggests distilled monoglycerides as a promising structurant to produce low saturated fat oil formulation for sandwich biscuits filling fat via oleogelation technique.

**Effect of hydrocoloids on the physicochemical properties of W/O emulsions reduced in fat.** Elena Dibildox-Alvarado, Universidad Autonoma de San Luis Potosi, Facultad de Ciencias Quimicas, Mexico; Marisol Cordova-Barragan, Universidad Autonoma De San Luis Potosi, Facultad De Ciencias Quimicas, Mexico; Jaime Pérez-Martínez, Universidad Autonoma De San Luis Potosi, Mexico

Currently the food emulsion industry has focused on developing products reduced in fat, which would imply an increase in the proportion of the aqueous phase, which compromises the stability of said emulsions, causing limited acceptance in the market. In this regard it is well known that the use of hydrocolloids (eg, pectin and xanthan gum), dispersed in the aqueous phase, can improve the stability of emulsions. High ester (HE) pectin or xanthan gum at concentrations of 0.2 and 0.3% w/w were added to the aqueous phase of a fat-reduced water-in-oil (W/O 35/65) emulsion for its application in bakery margarines, whose traditional fat content is 80%. The crystallization kinetics, interfacial tension, solid content (SC), and hardness were studied and compared with those obtained in a traditional W/O emulsion with 80% fat. Differential scanning calorimetry results showed a decrease in the crystallization enthalpy of the low melting triacylglycerols of the emulsion using xanthan gum, showing that this hydrocolloid could be migrating from the aqueous to the oil phase of the emulsion. Interfacial tension between both phases of the emulsion was not modified by the addition of HE pectin, but the xanthan gum caused an increase, showing an increment in the interfacial activity, probably because of the migration to the oil phase. A significant increase in the solid content and hardness was observed because of the increase in viscosity provided by the addition of these hydrocolloids. In conclusion, the reduced fat W/O emulsion added with xanthan gum in the aqueous phase matched the solid content and improved the hardness of the traditional W/O emulsion with 80% fat, which is interesting as it opens the possibility of generating healthier stable food products.

**Effect of interfacial composition on the rheological and digestion behaviour of emulsions.** Kunal Kadiya, University of Saskatchewan, Canada; Manisha Sharma, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

The rheological behaviour of emulsion can be tuned either through random jamming of droplets or the formation of a network of aggregated droplets leading to the structure formation in many food applications. The random jamming in nanoemulsion, at relatively low oil concentration, could be induced by decreasing the droplet size to nanoscale and increasing the interfacial shell-layer thickness. In this work, the interfacial shell-layer thickness was modulated by electrostatic deposition of positively charged chitosan (degree of deacetylation, DDA50% and DDA93%) on negatively charged Citrem (citric acid esters of mono-and-diglycerides)-stabilized nanodroplets. The aim was to determine the influence of interfacial shell-layer composition on the rheology and digestibility of emulsions at different concentrations (0–0.25wt%) of chitosans. With an increase in chitosan concentration and DDA, the negative charge of Citrem-stabilized droplets shifted to zero at 0.075wt% and reached a maximum at 0.2–0.25wt% chitosan. Correspondingly, the droplet aggregate size increased initially due to bridging flocculation till 0.1wt% and started decreasing at surface saturation around 0.2wt% chitosan. The gel strength of emulsions showed a similar trend with a significant increase up to 0.1wt% chitosan and decreasing thereafter and forming a stable viscoelastic gel at 0.2wt%. However, a further increase in chitosan concentration (0.25wt%) led to weak gels. The repulsive gelation in bilayer emulsions was attributed to an increase in the interfacial thickness by chitosan due to the formation of a strong steric interfacial layer on the Citrem-stabilized nanodroplet. The increase in shell-layer thickness was found to be a controlling factor for lipid digestion, where completely covered droplets (0.25wt% chitosan) showed lower lipolysis compared to zero or lower concentration of chitosan.

Such a change in the interfacial composition, to produce bilayer emulsions with tailored rheological and digestion properties, can be used as an attractive option to lower fat and deliver bioactives in emulsion gel-based foods.

**Effect of phase behavior of a binary blend of waxes used as organogelator on textural properties of vegetable oils-based organogels.** Natalia Martínez, Fac de Quimica (UDELAR), Uruguay; Bruno Irigaray, UDELAR, Uruguay; Ivan Jachmanian, UDELAR, Uruguay

Combinations of different waxes can allow us to design materials with specific physical properties for some food applications.

The objective of this work was to investigate the phase behavior of a binary blend of beeswax (BW) and carnauba wax (CRW) using a differential scanning calorimetry (DSC) Shimadzu 60A Plus. Further the textural properties of extra virgin olive oil (EVOO) and high oleic sunflower oil (HOSFO)-based organogels prepared with BW, CRW and a binary blend of BW/CRW, were studied.

The binary system was prepared by mixing BW/CRW in different proportions from 0 to 100 % in 10 % increments. Blends were crystallized in a DSC, and their melting behavior was used to build a binary phase diagram. As result, depression in the melting temperature of the 70:30 BW/CRW mass ratio mixture was observed, suggesting the presence of an eutectic.

Vegetable oils-based organogels were prepared using BW, CRW and their mixture BW/CRW 70:30 ratio (MEF), at concentrations of 3, 4 and 5 %. Hardness was measured in a Agrosta 2 texturometer equipped with a 45 ° cone geometry probe at room temperature.

Results showed that oleogels prepared with 4 and 5 % MEF presented significantly higher values than those of pure waxes and 3% MEF (all of them less than 37 cN). Both EVOO and HOSFO-based oleogels showed 78 and 162 cN and 65 and 124 cN for 4 and 5% MEF, respectively.

Interactions between different waxes in the eutectic blend had a direct impact on the textural properties of these systems. It made possible to obtain vegetable oils-based organogels with a significantly higher hardness than pure waxes at the same concentration.

This research work is part of my PhD career in Chemistry, it includes a topic of great interest for my developments as a Researcher

**Fractionation of beeswax for edible oleogel applications.** Roman Sobolev, Federal Research Center of Nutrition, Biotechnology and Food Safety, Russia; Yuliya Frolova, Federal Research Center of Nutrition, Biotechnology and Food Safety, Russia; Varuzhan Sarkisyan, Federal Research Center of Nutrition, Biotechnology and Food Safety, Russia; Alla Kochetkova, Federal Research Center of Nutrition, Biotechnology and Food Safety, Russia

Edible oleogels are being developed as a promising way to reduce the content of saturated and trans-isomeric fatty acids in foods. One of the most studied classes of oleogelators is waxes. Waxes are mixtures of low molecular weight gelling agents, including hydrocarbons, wax esters, free fatty acids, and alcohols. These compounds can act as structuring agents either individually or in combination. The main objective of this work was the fractionation of beeswax with food-grade solvents to produce edible oleogels with specified properties. Fractionation was performed using preparative flash chromatography; hexane, acetone, and isopropyl alcohol were used as eluents. The resulting fractions were characterized using the following analytical methods: FTIR spectroscopy, HPLC-ELSD, TLC, SPME-GC-MS. Analysis of the chemical composition of the obtained fractions showed that the developed method allows obtaining fractions containing up to 94.8 % of hydrocarbons, 97.6 % of waxy monoesters, 98.7 % of the mixture of mono-, di- and triesters, as well as 82.6 % and 39 % of free fatty acids and alcohols, respectively. SPME-GC-MS analysis showed that the fractionation of wax by the proposed method allows obtaining fractions with different volatile compound profiles. The initial beeswax had no predominant component in the volatile compounds profile and was characterized by ‘‘floral’’, ‘‘aldehyde’’, ‘‘rose’’, and ‘‘citrus’’ descriptors. The fractions’ aroma profile, in turn, is characterized by descriptors ranging from ‘‘waxy’’, ‘‘fresh’’, ‘‘greasy’’, ‘‘mild’’ to ‘‘pungent’’, ‘‘solvent’’, ‘‘earthy’’. In most cases, one or more predominant components that determine the volatile substance profile are distinguished in the fractions. The results of the study showed that the proposed fractionation method can be used to obtain gelling agents from beeswax, which could potentially be further used in edible oleogels. Combining fractions with different chemical compositions will make it possible to develop oleogels with tailored properties.

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**Multi-scale approach to study crystallization behavior of cocoa butter-copra oil blends.** Julie Bloquet-Maurras, Centre de Recherche Paul Pascal, UMR 5031, Université de Bordeaux, CNRS, France; Mathilde Bayard, SOREDAB, France; Véronique Schmitt, Centre de Recherche Paul Pascal, UMR 5031, Université de Bordeaux, CNRS, France

The aim of this study was to characterize the changes induced in the crystallization of cocoa butter by the addition of different proportions of copra oil. To do so, cocoa butter (CB) and copra oil (CO) were blended in binary systems. Several fat blends with different ratios of CB/CO were crystallized at 4°C and then characterized using various techniques. Phase behavior diagrams were first constructed from the nuclear magnetic

resonance results. A multi-scale approach was used to study blends' physical properties such as microstructure and textural properties. The molecular diversity within the fat system was investigated. The microstructure of the fats was determined using a polarized light microscope. Textural properties were examined by rheological measurements at high deformation, using cone penetrometer. Solid fat content (SFC) profiles and isosolid diagrams of CB/CO mixtures showed eutectic formation, indicating a softening of cocoa butter by copra oil addition. The greatest extent of incompatibility occurs at the 60% copra oil addition level in 40% cocoa butter at 20°C. Crystal morphology and network's structure of blends were different as a function of the fat composition. Considering the textural properties, blends with high copra oil contents showed lower hardness in accordance with lower SFC eutectic effect.

**Oleoyl chloride succeed to efficiently esterify 4,4-dimethylsterols.** Liangliang Xie, Jiangnan University, China (People's Republic); Liyou Zheng, Anhui Polytechnic University, China (People's Republic); Tao Zhang, Jiangnan University, China (People's Republic); Dan Xie, Anhui Polytechnic University, China (People's Republic); Xingguo Wang, Jiangnan University, China (People's Republic); Qingzhe Jin, Jiangnan University, China (People's Republic)

Justification: Phytosterols have attracted increasing attention due to their excellent health benefits. However, free phytosterols possess high melting point and low solubility, resulting in their applications limited. Although 4,4-dimethylsterols and 4-desmethylstrerols are both phytosterols, the 4-desmethylstrerol esters could be synthesized using fatty acid and 4-desmethylstrerol by enzymatic method, and 4,4-dimethylsterol esters could not be. The carboxylic acids could be converted into the corresponding acyl chlorides, which are extremely reactive electrophiles and are well known to attack by alcohol nucleophiles to form carboxylic esters.

Objective: Therefore, the main objective of this study was to develop a suitable method for highly efficient synthesis of 4,4-dimethylsterol oleates using acyl chloride esterification.

Methods: The 4,4-dimethylsterol conversion was determined using high performance liquid chromatography (HPLC) equipped with a refractive index detector (RID). The synthesized products were then separated by flash chromatography. Subsequently, the structures of the 4,4-dimethylsterol esters were analyzed through attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy and ultra-performance liquid chromatography system coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS).

Results: The reaction conversion increased with increasing reaction time, temperature, and the molar ratio of oleoyl chloride to 4,4-dimethylsterols. The 4,4-dimethylsterol conversion of 99.27% was obtained with molar ratio of 1.1:1 at 313 K for 60 min. A second-order kinetic model was successfully developed for describing the esterification reaction. Arrhenius-Van't Hoff plot suggested activation energy and pre-exponential factor were 15.54 kJ.mol−1 and 1.78×103 L.mol−1.min−1, respectively. Structure analysis by ATR-FTIR spectroscopy and UPLC-Q-TOF-MS revealed 4,4-dimethylsterols was successfully converted into 4,4-dimethylsterol oleates.

Significance of your research to the AOCS membership: The study indicates that esterification through acyl chloride method is an adoptable approach for highly efficient production of 4,4-dimethylsterol oleates. The experimental results provide a valuable theoretical basis for the determination of the production process and large-scale production of 4,4-dimethylsterol oleate.

**Oxidative Depolymerized Nanocelluloses for Pickering Emulsion.** Li Ziqian, Aarhus University, Denmark; Zheng Guo, Aarhus University, Denmark; Oliver Bogojevic, Aarhus University, Denmark

As the awareness and social concern for the environment continuous to grow, is the interest for environmental-friendly materials getting more and more attention. Cellulose is the most abundant polymer in the world and its properties is well characterized and utilized in many different fields. Nanocellulose, depolymerized from cellulose, exhibits unique physical and chemical properties such as high specific surface area and high degree of hygroscopicity, which show great potential for applications in Pickering emulsions. In this work, we studied the Tempo-mediated oxidation carried out to depolymerize cellulose into nanocellulose and investigated its potential application in Pickering emulsions. Nanocellulose was successfully produced by Tempo-mediated oxidation, which was confirmed and analyzed by both Fourier-transform infrared spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). Transmission Electron Microscope (TEM), Contact Angle and Degree of oxidation (DO) of the nanocellulose were also investigated to obtain a detailed map… The contact angle of nanocellulose was lower than 90°, indicating that the nanocellulose had good wetting properties (hydration of the non-polar surface) and showed interfacial activity between oil and water. Different concentrations of the produced nanocellulose were used to investigate the effect on the stability of the O/W Pickering emulsions, with different oil contents. With the increment of the concentration of nanocellulose, the particle size of the emulsion decreases and the storage stability enhanced. On the other hand, with oil content increasing, the drop size of the emulsion increased but the stability increased. A total concentration of 0.9 wt% of nanocellulose could stabilize concentrated Pickering emulsion with 50 wt% of oil and had good storage stabilization for up to two weeks.

**Polymeric stabilized oleogel-based emulsions aiming at food lipid profile tailoring.** Artur Martins, International Iberian Nanotechnology Laboratory, Portugal; Ana Guimarães, University of Minho, Portugal; Pablo Fuciños, International Iberian Nanotechnology Laboratory, Portugal; Lorenzo Pastrana, International Iberian Nanotechnology Laboratory, Portugal; Pedro Sousa, Porminho, Alimentação, S.A, Portugal; Armando Venâncio, University of Minho, Portugal; Miguel Cerqueira, International Iberian Nanotechnology Laboratory, Portugal

Decisive processing and technological requirements like saturated fat reduction and healthier fatty acids delivery are immediate objectives of the food industry. In this work, our main focus was the development of gelled O/W emulsions, using oleogels as filling particles, that could act as healthier fat substitutes, namely for meat-based products. Glycerol monostearate (GM) was used as oleogelator of edible oils (linseed and sunflower oil), which were used to provide different fatty acid compositions to the resultant gelled emulsions. The oleogel fraction, containing nonionic surfactant polysorbate 80, was dispersed in an aqueous κ-carrageenan (3% wt.) solution and mixed at room temperature during 10 min. Oleogel-based emulsions exhibited suitable oleogel dispersion and complete gelling ability for compositional ratios ranging from 7:3 up to 9:1 (W:O) for samples produced with sunflower oil and from 6:4 to 9:1 (W:O) using linseed oil. The microstructural overall packing of the filling oleogel particles was enhanced for samples with higher aqueous ratios that consequently affected textural results. On the other hand, higher fractions of oil phase result in phase separation. The lipid profiles of oleogels were similar to those of the oils (the same as reported by the manufacturers), displaying levels of unsaturated fatty acids (UFA) above 80%, while oleogel-based emulsions exhibited total lipid content analogous to the percentage of oleogel incorporated in oleogel-based emulsions (approx. 40, 30, 20 or 10 %). Hardness decreased with higher oil phase ratios. The hardness values ranged from 4.5 (6:4) to 12.5 N (9:1) for linseed oil-based structures and between 6.5 (7:3) and 11.9 N (9:1) when sunflower oil was used. In conclusion, oleogel-based emulsions can be proposed for texture tailoring, fat reduction and bioactive delivery in processed foods.

**Profiling volatile composition of semi polar oleoresin fraction extracted from Syzygium cumini flesh and seed.** Malinda Madhusankha, Wayamba University of Sri Lanka, Sri Lanka; Chamila Jayasinghe, Wayamba University of Sri Lanka, Sri Lanka; Chamodini Thilakarathna, Wayamba University of Sri Lanka, Sri Lanka; Niranjala Perera, Wayamba University of Sri Lanka, Sri Lanka

Black plum (Syzygium cumini) is an underutilized functional fruit grown in the tropical regions, prominently in Indian subcontinent. The therapeutic effect in lowering blood sugar level, high antioxidant capacity, bright purple color and the unique fragrance of S. cumins fruit is highlighted its potential commercial applicability. Present study investigates main volatile components of flesh and seed of semi polar oleoresin fraction of S. cumins in order to enhance the shelf life and thermal stability in food applications. Essential oil of freeze-dried flesh and seed of S. Cumin was removed by hydro distillation and residue was used for extraction of oleoresins by soxhelt mechanism with ethanol. The profiling of oleoresin was done using GC-MS coupled with non-polar column and compounds were identified through NIST mass spectral library. Oleoresin yield of flesh is 2.86% and 23 volatile compounds were identified while seed were given 3.02% yield and 18 volatile compounds were identified. The prominent compounds in flesh were glycerone (12.13%) and dl- glyceraldehyde (8.89%) where glycerone is a derivative of glycerin with a sweet taste. Three types of succinate esters were found (5–6%) and they impart a natural flavor to the S. Cumin oleoresin which are useful in beverage manufacturing. The oleoresin obtained from S. Cumin seed comprises with 3.17% of palmitic acid which delivers anti diabetic properties, but excessive consumption may lead to deposit as low density cholesterol. The most abundant compound of seed oleoresin was delta- cadinine (9.11%) and it exhibits antimicrobial properties. Results conclude that semi polar oleoresin fraction of S. Cumin seed is suitable for therapeutic application while oleoresin fraction of flesh may useful in flavor generation in food application.

**Protein-ligand docking as a tool to predict properties and performance of plant-protein based bioplastics films.** Kristen Patnode, North Dakota State University, USA; Sara Johnson, Winona State University, USA; Andriy Voronov, North Dakota State University, USA; Bakhtiyor Rasulev, North Dakota State University, USA; Zoriana Demchuk, Oak Ridge National Lab, USA

Use of computational models to predict experimental outcomes within food packaging is a new approach and can serve to expedite advances in the field. Studies dedicated to bioplastic film formation have been performed for more than a century. By utilizing computational models, the amount of time and resources required in the wet-lab can be reduced, providing an economically effective means to further develop bio-based food packaging alternatives.

This study applies a combined computational and experimental approach, while focusing on plant protein-based films, which are emerging at the forefront of functional food trends. Protein-ligand docking approach was applied to model the intermolecular (non-covalent) interactions of select renewable additives with plant proteins, to demonstrate the effect of the incorporated

modifiers on properties of protein-based films. To support the molecular docking, we performed a set of experiments and successfully prepared films based on soy protein and zein protein which exhibit promising physical and mechanical behavior. By incorporation of natural plasticizers (glycerol and sorbitol) and reinforcement agents (micro-fibrillated cellulose) into the protein systems, films with more advanced mechanical properties and enhanced surface hydrophobicity were prepared, which confirmed the initial computational evaluations. In result, we found that computational protein-ligand docking approach can be used as an effective and accurate method in estimating the physical and mechanical properties of a film upon incorporation of modifiers into the plant-protein based system.

**Relationship Between Butter Physical Properties and Water Loss in a Laminated Pastry Model System.** Annalisa Jones, Utah State University; USA; Silvana Martini, Utah State University, USA

Butter is commonly used to produce laminated pastry since it provides ideal flavor and texture in the final product. However, during the processing of laminated pastry the butter structure can break due to shear stress, and water present in the form of a water-in-oil emulsion can be released from the system causing less flakiness in the dough. There is a need to unveil the physical properties that drive water loss in butter during lamination. Therefore, the objective of this study is to characterize the physical properties of 10 commercial butter products and correlate these properties to water loss during lamination. Samples were purchased from a local store and stored at 5°C for 24 h prior to testing. The physical properties evaluated include solid fat content (SFC), water content, water droplet size, viscoelastic behavior, melting profile, hardness, and water loss. A negative correlation was identified between water loss and SFC at temperatures of 30°C ($p=0.035$, $r=-0.685$) and 35°C ($p=0.015$, $r=-0.758$). Melting profiles of the samples were characterized by two melting peaks identifying medium-melting-fraction (MMF) and high-melting-fraction (HMF) triacylglycerols. The MMF enthalpy calculated from the melting curve was negatively correlated with water loss ($p=0.001$, $r=-0.915$). The HMF onset temperature ($p=0.002$, $r=-0.879$) and peak temperature ($p=0.006$, $r=-0.818$) also had a negative correlation with water loss. Finally, water loss was negatively correlated to hardness measured at 5°C ($p=0.017$, $r=-0.745$). None of the other physical properties showed a significant correlation with water loss ($p > 0.05$). Overall, this study shows that water loss can be reduced by formulating butters with high SFC at 30 and 35°C, high enthalpy of the MMF melting peak, high onset and peak temperature of the HMF peak and by increasing the hardness.

**Vegetable and Mineral Oils Organogels Developed by Mixtures of Phospholipids and Monoglycerides.** Jorge Toro-Vazquez, UASLP-FCQ, Mexico; Maria Bello-Santillan, Universidad Autónoma de San Luis Potosí, Mexico; Anaid De la Peña-Gil, Universidad Autónoma de San Luis Potosí, Mexico; Mayra Aguilar-Zárate, Universidad Autónoma de San Luis Potosí, Mexico; Miriam Charó-Alonso, Universidad Autónoma de San Luis Potosí, Mexico

In vegetable and mineral oils, the monoglycerides (MG) develop oleogels forming inverse bilayer structures stabilized by hydrogen bonds ($L\alpha$ phase). The $L\alpha$ phase is thermodynamically unstable resulting in the development of the $\beta$ polymorph followed by crystals' agglomeration. These processes affect negatively the oleogels' thermomechanical and oil-binding properties. In an attempt to stabilize the $L\alpha$ to $\beta$ transition we studied the MG and lecithin (LC) interactions in the development of mixed vegetable (VO) and mineral (MO) oil oleogels (15°C) using different MG to LC molar ratios (MG/LC between 1.1 and 8.1) at a constant total mass of gelators (2% and 4%). Independent of the total mass of gelator the crystal mass developed in the VO decreased as the MG/LC increased, while in the MO remained constant. The VO oleogels achieved its highest $G'$ at 8.1 MG/LC while in the MO at 1.1. The DSC and polarized light microscopy results showed that MG and LC have a solvent dependent synergistic interaction, developing oleogels with $G'$ that depended on the crystal 3D organization rather than on the crystal mass. At 8.1 MG/LC we observed that water addition (2.5 and 5 X 10–3 Moles of water/Mole of total gelator) increased the oleogels $G'$ ($P < 0.05$), and delayed the $L\alpha$ to $\beta$ transition, particularly in MO. Nevertheless, the water's pH (between 4 and 10) showed limited effect on the oleogels $G'$ providing the highest elasticity at $pH=4.0$ in both oils ($P < 0.01$), and at $pH=8.0$ just in the MO ($P < 0.05$). These results showed that through different MG/LC ratios and addition of small water volumes, we can develop MG-PL oleogels at lower total gelator concentrations than the one needed by MG or LC. Additionally, MG-PL oleogels had higher stability toward the $L\alpha$ to $\beta$ transition than that observed by MG oleogels.

**Viscoelastic improvement of water-in-oil emulsions for food and related applications.** Maria Romero-Pena, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

In this study, the increase in water content was investigated to improve the viscoelastic behaviour of water-in-canola oil (W/O) emulsions. Six sets of emulsions were prepared with high-pressure homogenization at > 60 ºC using different water fractions (20 – 50 wt%) containing 1.5 wt% LMP, and the oil phase containing glycerol monooleate (GMO) having same water-GMO ratio. All emulsions contained the identical concentration of

hydrogenated soybean oil (HSO) in the oil phase (7 wt %) to structure the W/O emulsions. All LMP-based emulsions remained stable after 30 days at 4°C, with no phase separation and flow behaviour than the control emulsions without LMP. Higher emulsion stability was observed with 40 and 50 wt% water than the emulsions with lower water content. All emulsions increased storage moduli proportional to water content rise without significant change between days 1 and 30, except for LMP-emulsions with 50 wt% water content. Water content augments the cluster size as more water droplets were packing together, forming a network and increasing gel strength. LMP at the interface also allowed the water droplets to weakly flocculated, preventing coalescence. Although all LMP-emulsions kept $G' \geq G''$ showing elastic behaviour, control emulsions showed $G'' \geq G'$ indicating viscous behaviour, suggesting that the presence of LMP is critical for droplet stability and emulsion gelation. Based on the appearance of bulk or emulsified water crystallization peak in differential scanning calorimetry (DSC). LMP-emulsions were completely stable at low water content (20 wt%) while partially stable at higher water content (30 – 50 wt%). However, control emulsions showed only bulk water peak, indicating unstable emulsions. Rheology, DSC, and emulsion stability test showed that increasing water droplet network and LMP interaction at the interface enhanced the stability and elasticity of W/O emulsions, which could lower fat content in spread foods products.

## Health and Nutrition

*Division Vice Chair: Matthew Miller, Cawthron Institute, New Zealand*

## COVID 19 Infections and Lipid Mediators: A Clinical Perspective

*Chair Charles Serhan, Brigham & Womens Hospital-Harvard Medical School, USA*

**Bioactive Lipids and Environmental Impact in COVID-19 Infections.** Darryl Zeldin, National Institutes of Health, USA; Matthew Edin, NIH, USA
Severe coronavirus disease 2019 (COVID-19) is characterized by pulmonary inflammation and induction of a systemic ‘‘cytokine storm’’ which includes release of proinflammatory cytokines such as interleukin-1β, interleukin-6, tumor necrosis factor-β; and interferonγ; which contribute significantly to morbidity and mortality. Preventing the severe local and systemic inflammatory response in COVID-19 may be as important as antiviral therapies. Endogenous lipid autacoid mediators play a critical role in inflammation and cytokine production/release. Cytochromes P450 can metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs), which have potent anti-inflammatory effects. These anti-inflammatory effects are reduced by soluble epoxide hydrolase (sEH)-mediated hydrolysis of EETs to less biologically active diols. Treatment with EETs or sEH inhibitors (sEHi) activate anti-inflammatory processes and may attenuate the cytokine storm. sEHi treatment attenuates acute lung injury in animal models of bacterial sepsis; however, little is known about the role of EETs or sEH inhibition in viral inflammation or COVID-19 disease progression. Current work is investigating the potential for sEHi treatment to attenuate inflammation in a human epithelial air-lung interface model of coronavirus infection and reduce morbidity and mortality during in vivo SARS-CoV-2 infection in mice.

**Covid-19 Patients and Lipid Mediators: Eicosanoids and Resolvins in the ICU.** Bruce Levy, Brigham & Womens Hospital-Harvard Medical School, USA
Acute lung inflammation is fundamentally important to host defense, but excessive inflammation can lead to critical illness including severe pneumonia, sepsis and acute respiratory distress syndrome that require treatment in an intensive care unit. The current pandemic of Severe Acute Respiratory Syndrome coronavirus-2 infection (aka coronavirus disease 2019 (COVID-19)) evokes excessive and maladaptive inflammation in a subset of patients leading to excess morbidity and mortality. Severe COVID-19 typically develops during the second week of infection as host inflammatory responses increase and viral titers decrease. Few treatments significantly mitigate COVID-19 severity; however, the RECOVERY trial demonstrated efficacy for dexamethasone as a host-directed therapy during this phase of COVID-19. In addition to dexamethasone, aspirin may also have protective actions for severe COVID-19. Both dexamethasone and aspirin modulate arachidonic acid metabolism and production of pro-inflammatory lipid mediators. In addition to their inhibition of prostaglandins, these interesting drugs can increase production of counter-regulatory and pro-resolving mediators, including annexin A1 and specialized pro-resolving mediators (SPMs). Bacterial or viral sepsis and acute respiratory distress syndrome represent life-threatening conditions secondary to unrestrained inflammatory responses in which these counter-regulatory mechanisms are defective. SPMs decrease the severity and enhance survival in murine model systems of pneumonia and sepsis. Targeted metabololipidomics have uncovered dysregulated lipid mediator abundance in sepsis with increased pro-inflammatory prostaglandins and leukotrienes and relative decrements in SPMs, suggesting important roles for lipid mediators in the pathogenesis of critical illness.

Here, we will present our clinical experience with severe COVID-19, latest research findings on severe pneumonia, sepsis and acute respiratory distress syndrome as well as potential applications to severe COVID-19 pathogenesis and potential therapeutic approaches.

**Inflammation Resolution: A Dual-pronged Approach to Mitigating Cytokine Storms in COVID-19 and Cancer?** Dipak Panigrahy, BIDMC-Havard Medical School, USA

Severe coronavirus disease (COVID-19) is characterized by pulmonary hyper-inflammation and potentially life-threatening ‘‘cytokine storms’’. Controlling the local and systemic inflammatory response in COVID-19 may be as important as anti-viral therapies. Endogenous lipid autacoid mediators, referred to as eicosanoids, play a critical role in the induction of inflammation and pro-inflammatory cytokine production. SARS-CoV-2 may trigger a cell death (‘‘debris’’)-induced ‘‘eicosanoid storm’’, including prostaglandins and leukotrienes, which in turn initiates a robust inflammatory response. A paradigm shift is emerging in our understanding of the resolution of inflammation as an active biochemical process with the discovery of novel endogenous specialized pro-resolving lipid autacoid mediators (SPMs; see CN Serhan Nature 2014, Serhan Levy JCI 2018), such as resolvins. Resolvins and other SPMs stimulate macrophage-mediated clearance of debris and counter pro-inflammatory cytokine production, a process called inflammation resolution. SPMs and their lipid precursors exhibit anti-viral activity at nanogram doses in the setting of influenza without being immunosuppressive. SPMs also promote anti-viral B cell antibodies and lymphocyte activity, highlighting their potential use in the treatment of COVID-19. Resolvins stimulate pathological thrombosis and promote clot removal, which is emerging as a key pathology of COVID-19 infection. Thus, SPMs may promote the resolution of inflammation in COVID-19, thereby reducing acute respiratory distress syndrome (ARDS) and other life-threatening complications associated with robust viral-induced inflammation. While most COVID-19 clinical trials focus on ‘‘anti-viral’’ and ‘‘anti-inflammatory’’ strategies, stimulating inflammation resolution is a novel host-centric therapeutic avenue. Many COVID-19 patients may also undergo cancer therapy to reduce tumor burden via surgery or killing of tumor cells, yet this simultaneously creates debris that may stimulate inflammation and tumor growth. Thus, conventional cancer therapy is inherently a double-edged sword via pro-inflammatory eicosanoid and cytokine storms. Enhancing endogenous clearance of tumor cell debris via resolvins is also new therapeutic target that may complement cytotoxic cancer therapies such as surgery, chemotherapy and radiation. Importantly, SPMs are currently in clinical trials for other inflammatory diseases and could be rapidly translated for the management of COVID-19 and cancer via debris clearance and inflammatory cytokine suppression.

## Covid-19 and Lipids

*Chairs Philip Calder, University of Southampton, United Kingdom; Martin Hewison, University of Birmingham, United Kingdom*

**Clinical Research on Omega-3 Fatty Acids and Its Role in Treating or Preventing COVID-19.** Adam Ismail, KD Pharma / GOED Omega-3, USA

COVID-19 infections are characterized by a cascading series of inflammatory symptoms. Omega-3s have long been associated with anti-inflammatory and inflammation-resolving mechanisms. More than 25 clinical trials and other investigations have been launched since the beginning of the pandemic exploring the role of omega-3s in COVID-19 infections. Many of the studies have focused on a manipulation of the cytokine storm as a potential mechanism of action for improving the course of the disease. However, clinical research in COVID-19 studies presents many challenges, particularly with nutrients like omega-3s that are being compared to faster-acting drugs and treatments. Additionally, the rapidly changing nature of clinical care during a pandemic creates complications for omega-3 fatty acids, with the standard of care shifting to include pharmaceuticals focused on suppression of pro-inflammatory cytokines and creating complications for researchers at trial sites. One ongoing trial exploring the effects of an eicosapentaenoic carboxylic acid formulation has encountered many such obstacles that forced it to use an adaptive design.

**Omega-3 and Covid-19: Potential Protective Mechanisms.** William Harris, Fatty Acid Research Institute, USA

Omega-3 fatty acids (EPA and DHA) have the potential to reduce the adverse effects of Covid-19 infection. In epidemiologic studies, omega-3 fatty acid levels are inversely associated with multiple circulating inflammatory markers. For example, higher RBC EPA+DHA levels are linked with lower levels of 10 biomarkers (e.g., CRP, IL-6, ICAM-1, LpPLA2, and TNFr2). In intervention studies, EPA supplementation (3g/d for 10 weeks) significantly reduced the expression of TNFa from LPS-stimulated monocytes as did a similar dose of DHA which, in addition, lowered IL-6 and MCP-1. More important are effects on clinical endpoints. A meta-analysis of 12 RCTs of omega-3 treatment in 1280 ICU patients with acute respiratory distress syndrome reported a significant improvement in measures of blood oxygenation in the treated patients and strong trends ($p \leq 0.08$) for reduced ICU length of stay and duration of mechanical ventilation.

The potential mechanisms underlying these actions are multiple. EPA/DHA are substrates for the production of inflammation-resolving mediators which cannot be made if the parent compounds are not present. Higher EPA/DHA levels reduce arachidonic acid availability from which are made some pro-inflammatory oxylipins (certain prostaglandins and leukotrienes). Quite independently of the synthesis of these mediators, the presence of EPA/DHA in inflammatory cells blocks the activation of the key pro-inflammatory transcription factor, nuclear factor kappa B thus retarding the entire intracellular inflammatory cascade. Finally, when EPA/DHA are inserted into cell membrane phospholipids they disrupt lipid rafts so as to disassemble surface receptors that respond to incoming inflammatory signals. All of these actions together result in a muted "cytokine storm" (i.e., rapid over-production of cytokines) which, when it occurs in alveolar macrophages, can rapidly result in death from Covid-19.

**Polyunsaturated Fatty Acids, Their Lipid Mediator Derivatives and COVID-19.** Philip Calder, University of Southampton, United Kingdom

The membranes of cells of the immune system have a high content of the n-6 PUFA arachidonic acid (AA) relative to the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The balance of these fatty acids determines membrane properties, generation of intracellular signals, gene expression, cellular responses and production of lipid mediators. Hence the PUFA composition of immune cell membranes can influence immune responses and inflammatory processes. Many lipid mediators produced from AA, including a number of prostaglandins and leukotrienes, are recognised mediators of inflammation, although some do have anti-inflammatory effects. EPA and DHA are incorporated into immune cell membranes partly at the expense of AA and hence oppose the actions of AA and its derived mediators. Hence the combination of EPA and DHA has been shown to reduce inflammation. Specialised pro-resolving lipid mediators produced from EPA and DHA (resolvins, protectins, maresins) are anti-inflammatory and inflammation resolving. Therefore, administration of EPA and DHA may be effective in treating uncontrolled inflammation as in the case of severe COVID-19. Both enteral and intravenous EPA+DHA have been used in patients with severe acute respiratory distress syndrome. A number of trials of n-3 PUFAs for prevention and treatment of COVID-19 have been proposed. The rationale for these trials and any findings that are available will be discussed.

**Resolvins and Pro-resolving Mediators from n-3 Pufas in Infectious Inflammation.** Charles Serhan, Brigham & Womens Hospital—Harvard Medical School, USA

Persistent inflammation when uncontrolled can play pivotal roles in widely occurring chronic diseases, such as neurodegenerative diseases,vascular diseases, metabolic syndrome and many other diseases of public health concern including COVID-19 infections. For humans, the ideal response to challenge is a self-limited inflammatory response leading to complete resolution. The resolution phase is now recognized as an active process with the biosynthesis of a superfamily of chemical mediators that signal and stimulate resolution of the inflammatory response. These mediators produced mainly from n-3 PUFAs precursors are coined specialized proresolving mediators (SPMs). SPMs are mediators that also include some ω-6 arachidonic acid-derived mediators i.e., lipoxins, ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-derived resolvins, protectins and maresins. These novel immunoresolvents, their biosynthetic pathways and receptors promote resolution of inflammation, clearance of microbes, reduce pain and promote tissue regeneration. SPMs evoke their actions via surface receptors. This presentation will focus on our recent advances on SPM biosynthesis and functions in the regulation intravascular leukocyte responses of interest in infectious inflammation and coagulation. The SPM pathways and receptors could provide a basis for new approaches for treating infectious-inflammation-associated diseases via resolution pharmacology and precision nutrition.

**Vitamin D, Immunity and Covid-19.** Martin Hewison, University of Birmingham; United Kingdom

Vitamin D has well-recognised benefits for skeletal health, and vitamin D-deficiency is associated with the bone disease rickets. However, it is now clear that vitamin D can influence a much wider range of target tissues, and vitamin D-deficiency has been linked to a wide array of human health issues. Notably the major circulating form of vitamin D, 25-hydroxyvitamin D (25D), is activated by cells from the innate immune system such as macrophages and dendritic cells. The resulting hormonal 1,25-dihydroxyvitamin D (1,25D) can interact with intracellular vitamin D receptors expressed by macrophages, dendritic cells and many other immune cells to promote important immunoregulatory actions. Prominent amongst these is the induction of antimicrobial cathelicidin, beta-defensins, and stimulation of autophagy. Whilst these mechanisms have been well defined for bacteria, it is clear that similar responses may also play a key role in viral infections. Vitamin D has further benefits for immune function in that it has potent anti-inflammatory activity that may help to moderate the potentially damaging inflammatory consequences of adaptive (lymphocyte) immune responses to infection. By suppressing Thelper(Th)1 and Th17 lymphocyte immunity, whilst promoting tolerogenic, suppressive regulatory T cells, 1,25D appears to be a crucial factor in preventing the

'cytokine storm' that accompanies some infections. This may be particularly important for infection with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/Covid-19) where vitamin D may play a role in both protecting against initial viral infection, whilst also attenuating lung damage that may occur in patients hospitalized with this infection. This presentation will provide an overview of the mechanisms for vitamin D immunoregulation, and to place these into the context of ongoing association studies and supplementation trials. The overall aim will be to highlight the potential benefits of vitamin D supplementation as a public health strategy for improved immune health in the Covid-19 era.

**Vitamin E, Immune and Inflammatory Responses and Respiratory Infections.** Simin Meydani, JM USDA Human Nutrition Research Center on Aging at Tufts, USA

Despite the development of antibiotics and vaccines, respiratory infections are a continuing threat to humans, as demonstrated by previous pandemics of influenza and the current one of COVID-19. Older adults are particularly susceptible to respiratory infections and morbidity and mortality from them. The age-associated decline in cell-mediated immunity and an increase in inflammatory responses contribute to the higher susceptibility to and morbidity and mortality from respiratory infections. Vitamin E, a potent lipid-soluble antioxidant, found in higher concentration in immune cells compared to other cells in blood, plays an important role in regulating immune and inflammatory responses. Vitamin E deficiency impairs normal functions of the immune system and increases oxidative stress and inflammation in animals and humans, which can be corrected by vitamin E repletion. Although deficiency is rare, vitamin E supplementation above current dietary recommendations has been shown to enhance the function of the immune system, reduce inflammation, and risk of respiratory infections, particularly in the aged. The mechanisms responsible for the effect of vitamin E on the immune system and inflammation have been explored at molecular and cellular levels, and in pre-clinical and clinical intervention studies. Vitamin E modulates T cell function through directly impacting T cell membrane integrity, signal transduction, and cell division, and also indirectly by affecting inflammatory mediators generated from other immune cells. The studies have shown that the combined modulatory effect of vitamin E on cell-mediated immunity and inflammatory responses results in reduced incidence of respiratory infections and morbidity from them. The majority of studies have been conducted with alpha-tocopherol form of vitamin E. Further studies are needed to determine if vitamin E supplementation beyond recommended level would be protective against other respiratory infections such as COVID-19 as well as the efficacy of other forms of tocopherol. Supported by USDA Agricultural Research Service Agreement #58-1950-4–003.

## General Health and Nutrition

*Chairs Kacie Ho, University of Hawaii at Manoa, USA; Ignacio Vieitez Osorio, PEDECIBA Quimica-UdelaR, Uruguay*

**Chitin Nanocrystals Reduce Lipid Digestion and β-carotene Bioaccessibility: An in-vitro INFOGEST Gastrointestinal Study.** Hualu Zhou, University of Massachusetts Amherst, USA

Engineered nanoscale materials (ENMs) are being intentionally added to some food and beverage products to achieve novel or improved functional attributes. However, there is still a relatively poor understanding of the behavior of many types of ENMs within the human gastrointestinal tract (GIT). In this study, insights into the gastrointestinal fate of nature-derived chitin nanocrystals ("nanochitin") was obtained using a standardized in vitro digestion model (INFOGEST), which is now widely used by food and nutrition scientists. The results obtained with the INFOGEST model were compared to those obtained with another commonly used static in vitro digestion model. Moreover, the impact of nanochitin dose on the digestibility and bioaccessibility of model food emulsions was examined: β-carotene-loaded oil-in-water nanoemulsions stabilized by a non-ionic surfactant (Tween 80). In agreement with the results obtained using an earlier in vitro digestion model, we found that the presence of nanochitin reduced lipid digestion and β-carotene bioaccessibility. However, the magnitude of these effects was considerably less for the INFOGEST model, which was attributed to the higher levels of pancreatic lipases and lower levels of calcium ions used. Overall, this study shows that nanochitin may be used to retard lipid digestion (which may be useful for creating foods that induce satiety), but that it also suppresses carotenoid bioaccessibility (which may reduce the nutritional value of foods). Consequently, food formulators must carefully consider all the potential impacts of edible nanoparticles when using them as functional ingredients in foods.

**Correlation Between Extent of Digestion and Self-assembly of Lipids in Human Colostrum and an Emulsified Colostrum Lipid Mixture.** Syaza Binte Abu Bakar, Monash Institute of Pharmaceutical Sciences, Australia; Malinda Salim, Monash Institute of Pharmaceutical Sciences, Australia; Andrew Clulow, Monash Institute of Pharmaceutical Sciences, Australia; Adrian Hawley, ANSTO, Australia; Donna Geddes, University of Western Australia, Australia; Kevin Nicholas, Monash

Institute of Pharmaceutical Sciences, Australia; Ben Boyd, Monash Institute of Pharmaceutical Science, Australia
Justification: Colostrum is the primary enteral of diet of infants due to lipids and bioactive proteins that can stimulate the development of organs and prevent diseases in infants. However, colostrum is produced in minute quantities during postpartum and current alternatives only promotes the growth and not development of infants.
Objectives: This project aims to formulate an emulsified colostrum lipid mixture and understand the differences in the self-assembly of lipids during the digestion of human colostrum and this colostrum mimic mixture.
Methods: Human colostrum samples were digested under intestinal conditions. The in vitro digestion model was coupled to small-angle X-ray scattering at the Australian Synchrotron, enabling acquisition of phase formation as a function of extent of digestion. Emulsified colostrum-mimicking lipid mixtures were formulated by weighing known amounts of triglycerides and dispersing the lipids with a buffer.
Results: Prior to digestion, a lamellar phase was present in human colostrum caused by the formation of calcium soaps due to self-digestion by the breast milk's own bile salt-stimulated lipase (BSSL). In contrast, a lamellar phase was not evident in emulsified colostrum before the start of digestion due to the absence of BSSL and only grew once digestion was initiated. As the concentration of calcium in the digestion samples was increased, an additional non-lamellar phase and sponge phase were only seen for emulsified colostrum and not human colostrum. This difference could be attributed to differences in extents of digestion. However, as bile salt micelles were added to the samples, human and emulsified colostrum displayed same phases due to similar extents of digestion.
Significance of research: The formulation of an emulsified colostrum lipid mixture and monitoring the phase transition of lipids during digestion aid in the understanding of the ability of these colloidal structures to potentially deliver lipophilic nutrients to the systemic circulation of infants.

**Crystallinity of Emulsified Triacylglycerols Modulates Lipemic Response in Healthy Male Participants.** Amanda Wright, University of Guelph, Canada
Understanding how nuanced differences in dietary triacylglycerol (TAG) supramolecular structure and physical properties alter metabolic response may help to clarify the contributions of dietary lipids and specific fatty acids to health and disease. This includes the possible direct and indirect roles of TAG crystallinity. This talk will highlight the role of emulsion TAG crystallinity in modulating postprandial lipemia, mediated through differences in gastric structure and emptying. Plasma lipemic response was delayed when healthy men consumed compositionally identical palm stearin emulsions tempered to contain partially crystalline versus undercooled liquid droplets. Static in vitro digestion results confirmed the differences were both directly attributed to droplet physical state and indirectly to colloidal behavior in the stomach (i.e., resistant or susceptible to acidic flocculation). Definitively, all other things being equal, the presence of TAG crystallinity in a lipid-rich meal was shown to affect postprandial metabolism. Differences in emulsion gastric structure and emptying rate were subsequently evidenced by high-resolution ultrasound for a series of size-matched palm stearin and palm olein emulsions, designed to explore the contributions of TAG physical and gastric colloidal behavior to postprandial lipemia and satiety. The technique yielded qualitative insights relating emulsion physicochemical properties with metabolic response. The results also confirmed the augmenting influence TAG crystallinity has in delaying lipid stomach emptying, with implications for lipemia and satiety, following ingestion of a lipid-rich meal by healthy men. This work rationalizes greater consideration of how TAG physical state may have influenced the results of previous studies and be capitalized on to modulate metabolism.

**Effect of Agitation and Added Cholesterol Esterase on Bioaccessibility of Phytosterols in a Standardized In Vitro Digestion Model.** Abigail Boyd, Iowa State University, USA; Joey Talbert, Iowa State University, USA; Nuria Acevedo, Iowa State University, USA
The COST INFOGEST network of scientists and researchers have developed a standardized, static method of in vitro digestion to reduce costs and improve comparability across laboratories. The protocol is simplified and likely appropriate for most studies. However, for more complex studies such as nutrient bioaccessibility, the standardized protocol may be inadequate. In the present study, the INFOGEST protocol was evaluated for its efficacy in studying the bioaccessibility of phytosterols (PS). Particularly, the effect of manipulating different method variables was investigated. Soybean oil containing 2% of PS (free or esterified to fatty acids) was used as a test sample for each experiment. PS-soybean oil samples were digested according to the INFOGEST protocol without modification. Modifications included changing the agitation type from orbital shaking to head over heels (HOH) agitation, as well as the addition of cholesterol esterase (CE) during the intestinal phase. During digestion, free fatty acids (FFA) were quantified by GC to confirm the progress of lipid hydrolysis. PS bioaccessibility throughout digestion was measured by GC quantification of PS content in the micellar phase as a percentage of total PS. The INFOGEST protocol with no modification showed that free phytosterols (FPS) had a bioaccessibility of 14.6%. This was significantly greater than that of esterified phytosterols (EPS), which was 4.2%. Bioaccessibility of FPS significantly increased to

19.1% with HOH agitation, while there was no significant change in EPS bioaccessibility. At different concentrations of CE, there was no significant change in EPS bioaccessibility, likely due to steric limitations of CE on EPS. The results of this study indicate that the standardized INFOGEST protocol must be improved in order to perform more complex analyses. The type of agitation should be specified, especially for biphasic systems. In addition, the activity and specificity of enzyme extracts must be verified to be comparable to human digestion.

**Effect of Extraction Solvent and Technique on the Antioxidant and Antidiabetic Activity of Thebu (Costus Speciosus (j. Koenig) Sm) Rhizome.** Dumila Roshani, Wayamba University of Sri Lanka, Sri Lanka
Costus speciosus has long been used in traditional systems of medicine. The plant has been found to possess a diverse number of pharmacological activities. Therapeutic activity of bioactive compounds of plant material may vary with extraction conditions and determine its possible food applications. This work was compared the antioxidant and antidiabetic activity of C. speciosus rhizome that prepared as water, methanol and ethanol extracts via homogenization, sonication and maceration in order to determine its suitable food application. Water, methanol; pure solvent (99%), 95%, 90%, 80%, 50% and ethanol; 95%, 90%, 80%, 50% extracts of rhizomes were tested for DPPH radical scavenging activity, total antioxidant capacity (TAC) and alpha-amylase inhibitory activity.
Pure and 95% methanol extracts via maceration showed the highest significant ($p < 0.05$) percentage inhibition of DPPH radical which were $71.50 \pm 0.87\%$ and $73.11 \pm 0.65\%$ respectively. The highest TAC was observed in pure and 95% methanol extracts via maceration as $7.46 \pm 0.30$ and $7.36 \pm 0.06$ mg Ascorbic Acid Equivalent (AAE)/ g dry weight respectively. Pure methanol extract via maceration ($82.97 \pm 0.11\%$) showed the highest alpha-amylase inhibition activity.
The results revealed that vigour of the extraction procedure and efficiency of the extracting solvent were mainly affected to the amount of the bioactive compounds that can be extracted from the plant material as rhizome extracts prepared using methanol and maceration showed significantly higher results. However, water extract prepared from maceration showed a considerable amount of DPPH radical inhibition ($41.49 \pm 0.88\%$), TAC ($2.45 \pm 0.22$ mg AAE/ g dry weight) and alpha-amylase inhibitory activity ($9.34 \pm 0.46\%$). Hence, bioactive extracted from C. speciosus rhizome with water via maceration would be suitable to use in direct food applications to treat diabetes mellitus.

**Replicating the Self-assembly of Human Breast Milk Lipids During Digestion with Emulsions of Cow Milk Fat Mixed with Canola Oil.** Andrew Clulow, Monash Institute of Pharmaceutical Sciences, Australia; Malinda Salim, Monash Institute of Pharmaceutical Sciences, Australia; Syaza Binte Abu Bakar, Monash Institute of Pharmaceutical Sciences, Australia; Cameron Nowell, Monash Institute of Pharmaceutical Sciences, Australia; Adrian Hawley, ANSTO, Australia; Ben Boyd, Monash Institute of Pharmaceutical Science, Australia
Justification & Objective: Digestion of milk lipids in our intestines yields monoglycerides and fatty acids that self-assemble into a variety of liquid crystalline structures. This self-assembly process is species dependent, suggesting an important role for these structures in infant nutrition. Our recent work has focussed on designing lipid mixtures that replicate the self-assembly of different natural milks during digestion.
Methods: Lipid mixtures were prepared by mixing cow milk fat and canola oil. These lipid mixtures were dispersed using casein as an emulsifier or canola oil was mixed directly with cow's milk to generate milk-like emulsions. Coherent anti-Stokes Raman spectroscopy (CARS) microscopy was used to confirm mixing of the canola oil into cow's milk and small angle X-ray scattering with in situ lipolysis was used to measure the self-assembly of lipids in emulsions of the mixed lipids during digestion.
Results & Significance: The triglyceride composition of the digesting emulsions was found to be a primary driver of lipid self-assembly during milk digestion. By varying the amounts of emulsified medium/long chain saturated lipids in cow milk fat and long chain unsaturated lipids in canola oil, lipid mixtures could be produced that replicated both natural milks and infant formulae. The lipid self-assembly in emulsions containing a 1:1 weight ratio of cow milk fat and canola oil were found to replicate that of human breast milk, irrespective of whether the lipids were pre-mixed and emulsified or whether the canola oil was dispersed directly into cow's milk. These mixtures provide lipid compositions that can be used as milk mimics with tuneable colloidal structures to investigate the interactions of endogenous surfactants, lipophilic drugs and nutrients with milk in the gastrointestinal tract.

## General Health and Nutrition Awards

*Chairs Kacie Ho, University of Hawaii at Manoa, USA; Ignacio Vieitez Osorio, PEDECIBA Quimica-UdelaR, Uruguay*

**How Quantitative and Kinetic Approaches to Studying Brain Docosahexaenoic Acid Metabolism Led to New Methods, Some Answers, and a Lot of Questions.** Richard Bazinet, University of Toronto, Canada
This lecture will focus on translational studies in omega-3 metabolism that my lab has contributed to. Upon learning quantitative and kinetic approaches to study fatty acid metabolism with Stephen Cunnane

and Stanley Rapoport, I established a laboratory at the University of Toronto with a focus on these acquired skills. By studying the mechanisms of uptake of docosahexaenoic acid (DHA) into the brain in preclinical models, we were able to contribute to the first estimates of human brain DHA uptake rates and, thus, requirements in humans. Estimates of how much DHA the human brain needed led to new questions about how diet could supply the brain with DHA. We then developed quantitative steady-state infusion approaches to measure DHA synthesis from its dietary precursors in preclinical models. While we were able to translate our steady-state infusion models to humans, it was apparent that a less invasive method was needed. Recently we stumbled across natural abundance carbon isotope ratio analysis and developed methods to distinguish between DHA obtained from marine sources or its dietary precursors. Within just a few years of developing new methods, we were able to translate and confirm several of our preclinical findings to humans, including flux though the omega-3 pathway and limited retroconversion. This natural evolution of quantitative approaches in my research program has produced some answers and a lot of new questions.

**Human Nutrition and Health Viewed Through the Diversity of Fatty Acid Structures.** Tom Brenna, University of Texas at Austin, USA

Twenty-one amino acids are necessary and suffice to enable the assembly of all proteins from the simplest of prokaryotes to the most complex specialized human cells. In contrast, many hundreds of fatty acids are synthesized by cells and consumed in foods. The structural diversity of fatty acids can be very limited in complex cells, for instance straight chain and a single position desaturation in S cerevisiae, or highly complex in specialized cells of complex organisms, for instance straight, branched chain, unsaturated and polyunsaturated in mammalian sebocytes. Methods that reveal the richness of fatty acid structures provide key insights into many aspects of biology.

Our research group aims to understand human nutrition and health aided by development of novel, highly specific methods for rapid analysis of fatty acid methyl esters (FAME). Gas chromatography-combustion-isotope ratio mass spectrometry, originally developed for studies of natural variability, was coupled to highly enriched fatty acids for highly sensitive tracer analysis in humans and non-human primates. This technique enabled studies showing that in vivo synthesis of DHA from ALA precursor is below 1% in humans and non-human primates. Covalent adduct chemical ionization (CACI) tandem mass spectrometry enables rapid structural characterization of double bond position in conventional FAME mixtures. Recent results reveal dozens of novel fatty acid structures in cow's milkfat. Collisional dissociation of molecular ions generated by electron ionization provide unambiguous localization of chain branching, enabling characterization of branched chain fatty acids in vernix caseosa and similar secretions of California sea lions.

**Omics Technologies to Control the Formation of Lipid Oxidation Products in Food and Their Metabolic Effects.** Marc Pignitter, University of Vienna, Austria

Oxidation of dietary lipids may cause deteriorative changes in chemical, sensory, and nutritional food properties. Vegetable oils, for instance, are rich in polyunsaturated fatty acids and considered healthy, although these dietary fats are prone to oxidation. The limited shelf life of these products is determined by their low oxidative stability, which can be characterized by an accumulation of various lipid oxidation products that are supposed to have detrimental health effects. However, it is very common to rely on traditional methods focusing on selected analytes leaving unrecognized the majority of oxidized lipids. Applying omics approach allows to identify (i) matrix-dependent lipid oxidation products and potential markers, (ii) measures to increase the oxidative stability of food lipids during production and processing, and (iii) mechanistic insights into the progress of lipid oxidation in differently-treated foods by not only shedding light on a single class of oxidized lipids but providing a holistic view.

The emerging 'omics' technologies were also used to study the metabolic role of dietary oxidized lipids. It could be demonstrated that oxidized lipids from differently oxidized oils regulate phospholipid metabolism in gastrointestinal cells depending on their chemical structure. Similarly, structure-dependent effects were also observed for the fatty acid uptake, indicating that hexanal promoted fatty acid uptake while 13-HpODE showed no effect, raising questions about the potential role of dietary oxidized lipids in the development of inflammatory bowel diseases and other gastrointestinal pathologies. Most strikingly, the oxidized lipids-induced metabolic effects were shown on a genomic as well as metabolomic level. Integrated pathway analysis of the combined genomic and metabolomic effects revealed that also pathways of the amino acid and protein biosynthesis were affected by primary and secondary lipid oxidation products but not by the non-oxidized lipid.

## Health and Nutrition Division Student Eposter Pitch Competition

*Judges Fabiola Dionisi, Societe' des Produits Nestlé - Nestlé Research, Switzerland; Matt Miller, Cawthron Institute, New Zealand*

Sponsored by the AOCS Foundation and supported by the AOCS Health and Nutrition Division

**Effects of Dietary Phospholipids Prepared from the Japanese Giant Scallop (Patinopecten yessoensis)**

**on Cholesterol Metabolism in C57BL/6J Mice.** Koki Sugimoto, Kansai University, Japan; Taiki Shinagawa, Kansai Univ, Japan; Ryota Hosomi, Kansai University, Japan; Munehiro Yoshida, Kansai University, Japan; Kenji Fukunaga, Kansai University, Japan

Our previous study showed that dietary scallop oil (SCO), containing high eicosapentaenoic acid and phospholipids (PL), decreased the serum and liver cholesterol contents in mice. In the present study, we focused on PL in SCO (SCO-PL) and investigated the mechanism of its cholesterol-lowering effect using mice. Five-week-old male C57BL/6J mice were fed lard based high-fat and cholesterol diets containing 3% soybean oil (SOY-TG group), or soybean PL (SOY-PL group), or triglyceride (TG)-type oil with almost the same fatty acid composition as SCO-PL (SCO-TG group), or SCO-PL (SCO-PL group) for four weeks. SCO-PL diet significantly decreased the serum and liver cholesterol contents compared with SOY-TG diet. The serum and liver cholesterol-lowering effect of SCO-PL was partly mediated by the enhancements of the liver Cyp7a1, which is a rate-limiting enzyme for bile acid synthesis, expression level. On the other hand, the ileum farnesoid X receptor (Fxr) and fibroblast growth factor 15 (Fgf15) expression levels in the SCO-PL group were significantly decreased compared with SOY-TG group. Additionally, the liver β-muricholic acid (MCA) content and Cyp2c70, which synthesize chenodeoxycholic acid to MCA, expression level in mice fed SCO-PL were significantly increased compared to mice fed SOY-TG. From these results, the increase of the liver Cyp7a1 expression level by dietary SCO-PL in partly through the reduction of the ileum Fgf15 expression level due to the increase of ileum taurine-conjugated βMCA, which is an antagonist of Fxr. This study indicated that dietary SCO-PL can be a health-promoting component for reducing cholesterol content.

**Efficacy of dietary γ-glutamyl peptide in modulating vascular inflammation.** Snigdha Guha, University of Nebraska, Lincoln, USA; Kaustav Majumder, University of Nebraska-Lincoln, USA

Cardiovascular diseases (CVDs) accounts for around one-third of annual global deaths. Increased level of vascular inflammation contributes to the pathogenesis of various CVDs such as atherosclerosis. Dietary bioactive peptides have been shown to exhibit anti-inflammatory properties and could be used for the prevention or management of CVDs. Dietary γ-glutamyl valine (γ-EV), commonly found in dry- edible beans, reduced gastrointestinal inflammation via allosteric activation of Calcium-Sensing Receptors (CaSR). Thus, it was hypothesized that the intervention γ-EV might modulate the inflammatory responses in vascular endothelial cells via the activation of vascular CaSR. Human tumor necrosis factor-α (TNF-α) was used to induce inflammation in pre-treated (2h with γ-EV at 0.01mM, 0.1mM, and 1mM) human aortic endothelial cells (HAECs). TNF-α treatment significantly increased the expression of inflammatory adhesion molecules (VCAM-1, E-Selectin), chemokine (MCP-1), and cytokines (IL-6 and IL-8). However, the expression of these biomarkers was significantly downregulated by 1mM γ-EV pretreatment. Additionally, the anti-inflammatory effect of γ-EV was attenuated entirely by the intervention of selective CaSR inhibitor, NPS2143. Further investigation also led to the identification of certain key modulators, such as β-arrestin2, CREB, and p65, which were involved in the CaSR-dependent anti-inflammatory effect of γ-EV. The transport efficiency of γ-EV was evaluated through the monolayer of intestinal epithelial cells (Caco-2), and the apparent permeability (Papp) of the peptide was found to be 1.56x10–6 cm/sec. The mechanism of transport of the peptide across the Caco-2 cells was found to be both paracellular as well as PepT1-mediated. Thus, the study concluded that dietary γ-EV could significantly reduce vascular inflammation and can be used to develop functional foods or nutraceuticals for the prevention and management of CVDs.

**Tribo-rheological analysis of reconstituted gastrointestinal porcine mucus under different pH conditions.** Gustavo Ruiz-Pulido, Tecnologico De Monterrey, Mexico; Dora Medina, Tecnologico de Monterrey, Mexico

The project analyses the physicochemical and mechanical properties of pig gastrointestinal mucus from a rheological point of view. Considering that mucus is a viscoelastic protective barrier that covers the internal walls of gastrointestinal tissues, developing fundamental functions as: protecting of the body from the entrance of environmental pathogens and toxins, lubricating of the epithelium to reduce mechanical stress (shear strength) caused by swallowing food, avoiding excessive water loss to maintain hydration, and shielding the stomach from gastric acids. The viscoelastic and protective properties of mucus are mainly produced by a mucin network, stabilized through electrostatic, hydrophobic and hydrogen bonding interactions. Mucin is a glycoprotein with a polyanionic nature at physiological pH, which is determinant in mucus rheology. At neutral pH, mucus presents viscous behavior produced by chains crosslinking. Instead, at acidic pH, mucus acquires an extended conformation related to an elastic behavior produced by mucus gelation in the stomach. Therefore, rheological analysis is important to visualize changes in viscosity, friction coefficient, resistance to different shear stresses and oscillatory stress to which the intestinal mucus is subjected under natural conditions, considering the different pH values of the gastrointestinal.

**Uptake of fatty acids by the enterocyte: New insights gained from mathematical modeling.** Julie

Etienne, Inserm U1060, INRAE UMR1397, UCB Lyon 1, INSA-Lyon, Inria, France; Armelle Penhoat, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Marie-Caroline Michalski, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Carole Knibbe, Inserm U1060, INRAE UMR1397, UCB Lyon 1, INSA-Lyon, Inria, France
Justification: After a meal, fatty acids (FA) released from digested dietary triglycerides, are taken up by absorptive cells, namely intestinal enterocytes. This step is usually reviewed as being composed of passive diffusion along with a carrier-mediated pathway. However, in the absence of a clearly identified membrane carrier, it remains unclear whether the latter actually exists.
Objective: We aim at testing, using mathematical modeling and data fitting, whether the observed dose-response curves of apical enterocyte FA uptake are actually compatible with the pathways suggested by the literature. We propose relevant alternative mechanisms.
Methods: Uptake of FA by the enterocyte was first modeled as passive diffusion only (model M0) and studied theoretically. Two other models were implemented: the one supported by literature with both passive diffusion and a carrier protein (M1); an alternative one with passive diffusion followed by enzymatic transformation inside the cell (M2). We compared these models to experimental data from the literature on rodents and Caco-2 cells.
Results: The analytical study of M0 allowed us to (1) demonstrate that the dose-response curve depends much more on volume ratios than on permeability values, (2) predict the dose-response curve for a wide range of realistic volumes, and (3) show that even the highest values cannot produce a high enough uptake response. The active mechanisms added in M1 and M2 allowed the response to reach experimental data. Both M1 and M2 fitted the data equally well. However M2 has a much larger uptake potential for the same protein parameters, thereby suggesting that it is more efficient for the cell to invest in an intracellular enzymatic transformation than in a membrane carrier.
Significance to AOCS: Interdisciplinary, quantitative mathematical modeling may help targeting molecular candidates and guide experimental effort to gain insight on physiological mechanisms of importance in the health impact of dietary lipids.

## Teaching and Learning in the Time of COVID-19

*Chairs Nuria Acevedo, Iowa State University, USA; Tong Wang, The University of Tennessee, USA*

**Adapting Undergraduate Laboratory Curriculum in a Pandemic: Strategies for Introductory and Upper-division Labs.** Matthew Fhaner, University of Michigan–Flint, USA
Faculty from all over the world were required to modify their laboratory curriculum in early 2020 with minimal time to accommodate alterations. Since then, academia has struggled with how to provide a high-quality and impactful laboratory curriculum in the face of various university and government restrictions. A variety of strategies have been identified including in-person, remote, and hybrid learning modes for laboratory courses. In this presentation, undergraduate chemistry laboratories at a regional comprehensive university (The University of Michigan – Flint) will be highlighted. Specific course examples will include moving a traditional general chemistry lab 100% remote for the Spring 2020 term, and renovating two laboratory courses (general chemistry and quantitative analysis) to fit a hybrid instructional model for the Fall 2020 term. In addition to course overviews, specific challenges, successes, and reflections will be provided.

**CREATIVE Pivoting to Online Teaching.** Lilian Were, Chapman University, USA
This talk will address Changes in content delivery; Resources that were used to ensure Ethics, Active learning, fair assessment, Teamwork, and Interaction in the Virtual Environment of my Research Methods, Food Ingredients, and Food Chemistry courses. Conversion from in-person classes to remote teaching involved changes in assessment with more weight put on out-of-class assignments. To minimize contract cheating, limiting the exam time was done to ensure that students did not confer with others. Students also sign an honesty pledge at the beginning and end of the exams and quizzes and cameras while taking exams. Besides exams, assessment and active learning were achieved by giving students a choice between synchronous and asynchronous presentations. Students view the asynchronous presentations before class, and we used class time for question and answer. The shared student learning with tools such as google-drive helped raise everyone to the same high standards. Pre-class readings or video viewings enrich class discussions, but some students with great insights do not necessarily talk during the class discussions in the virtual space, and the use of google docs has been a way to hear from the shy and quiet students. These and other strategies used to ensure a rich learning environment amidst the change will be shared.

**Teaching and Learning in the Time of COVID-19.** Laura Bestler, Center for Excellence in Learning and Teaching at Iowa State University, USA
Coronavirus has thrust to the haste of remote teaching and learning, placing an unprecedented number of educators to teach using online platforms have little or no training to do so. As part of the PE-CIG's commitment to promoting excellence in teaching through discussion and exchange of ideas, we are sponsoring a Hot Topic

Session that will feature practical ideas and successful strategies for educators to use in a remote environment. This Hot Topic Session is geared toward our professional educators with the overall goal to foster inspiration and innovation and provoke conversations that matter in a teaching and learning environment where on-line instruction plays a major role. This session will consist of a Keynote speaker followed by two discussion sessions where professional educators will share their online teaching and learning experience. To conclude the session, the invited speakers will participate in a panel of Q&A where they will have the opportunity to interact and exchange ideas with the audience.

**Shift to On-line Food Chem Lab Conducting a Hybrid Class: An Experience.** Vermont Dia, University of Tennessee Knoxville, Institute of Agriculture, USA
The COVID-19 pandemic has led to a different teaching strategy in Spring 2020 and Fall 2021. This sharing of teaching experiences will focus on my Food Chemistry Laboratory class that was converted to a fully-online class right after the university decided to cancel in-person instruction in the last month of Spring 2020. My experience in preparing video materials for laboratory experiments on the kinetics of enzymatic browning and stability of vitamin c will be highlighted. Synchronous data analysis will also be discussed. In addition, I will share my experience of teaching an introductory course in food science involving an in-class activity using a hybrid format. I hope that the audience will get some tips from this sharing experience that can lead to a lively panel discussion.

**Strategies for the Assessment of Students Learning in Online Platforms.** Amanda Wright, University of Guelph, Canada
For many courses, the shift to remote learning necessitated changes in how student learning is assessed, both in the individual and group contexts. This involved creating or modifying assignments, using alternate technologies, and/or implementing other strategies to ensure and measure student success. Group work is an essential element of many undergraduate classes, and yet it can suffer when individual students are under high levels of stress. Recognizing the potential for widespread stress during the pandemic, we took extra steps to ensure positive peer-to-peer student interactions and success on joint assignments. The effectiveness of these and other course learning assessment strategies in NUTR4330 Applied Nutritional & Nutraceutical Sciences – II at the University of Guelph will be discussed.

**Teaching for Learning On-line: More Than Just Moving Course Notes to Virtual Platforms.** Karen Schaich, Rutgers University, USA
Teaching effectively on-line requires more than just presenting regular notes or slides over the internet. Students read notes on-line differently than on paper or a class screen, so formatting of notes must be changed for virtual teaching. Auditory learning is different than studying written texts. This talk will address how pictures and diagrams and more written details are needed to help students build picture sin their minds (in place of demos and samples in class), and how applications and extensions must be built in to lectures to connect students from theoretical to real life to keep students involved.

## The Effect of Omega-3 Dosage on Cardiovascular Outcomes

*Chair Doug Bibus, Lipid Technologies, LLC, USA*

**The Effect of Omega-3 Dosage on Cardiovascular Outcomes.** Aldo Bernasconi, GOED, USA
Despite significant advances in the prevention and treatment of cardiovascular diseases (CVDs), they remain the leading cause of mortality in the United States and most of the world. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, the two main omega-3 long-chain polyunsaturated fatty acids of marine origin, are widely used for the prevention of CVD outcomes, but randomized control trials (RCTs) have found inconsistent results. A recent meta-analysis of interventional trials presents strong evidence that EPA+DHA supplementation is an effective strategy for the prevention of certain CVD outcomes and that for certain outcomes the effect is dose-dependent. This presentation will present these results and address other possible causes for the heterogeneity of RCT results."

## Cannabinoid ABCs: The 411 on CBC, CBD, CBG, and THC (AOCS & ISSFAL Joint Session)

*Chairs Rinat Ran-Ressler, Nestle Health Science, USA; Holiday Durham Zanetti, Nutrilite Health Institute, Amway*

**Cannabinoid-terpenoid Synergy and the Entourage Effect.** Ethan Russo, MD, CReDO Science, USA
From 1964 when Raphael Mechoulam isolated and synthesized tetrahydrocannabinol, it has been the primary focus of cannabis research. More recently, the synergistic contributions of cannabidiol to its pharmacology and analgesic medicinal value have been demonstrated (1). Other phytocannabinoids including tetrahydrocannabivarin, cannabigerol, cannabichromene and the cannabinoid acids harbor additional effects of therapeutic interest. Innovative conventional plant breeding has yielded cannabis

chemotypes expressing high titers of each component for future study.

This presentation will introduce another echelon of phytotherapeutic agents, the cannabis terpenoids (2–4): limonene, myrcene, α-pinene, linalool, β-caryophyllene, et al. These half-siblings of phytocannabinoids are all flavor and fragrance components common to human diets that have been designated Generally Recognized as Safe (GRAS) by the US Food and Drug Administration and other regulatory agencies. Terpenoids are quite potent, and affect animal and even human behavior when inhaled from ambient air, at serum levels in the single digits ng/ml. They display unique therapeutic effects that may contribute meaningfully to the entourage effects of cannabis-based medicinal extracts.

Particular focus will be placed on phytocannabinoid-terpenoid interactions that could produce synergy with respect to treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, etc.

Phytocannabinoid-terpenoid synergy, if proven, increases the likelihood that an extensive pipeline of new therapeutic products is possible from this venerable plant.

**Increased Levels of Serum Palmitic Acid in Cannabis Users.** Giulia Cisbani, University of Toronto, Canada; Alex Koppel; Romina Mizrahi, Richard Bazinet, University of Toronto, Canada

Cannabis is now legal in many countries and while numerous studies have reported on the impact of cannabis on cognitive function and appetite regulation, metabolic processes in cannabis users (CU) use are still poorly understood. Thus, we conducted an exploratory analysis to evaluate whether cannabis use could alter fatty acid metabolism in CU ($n = 21$) compared to a group of healthy volunteers (HV, $n = 16$). Interestingly, we observed an increase in the serum levels of some saturated and monounsaturated fatty, including palmitic, palmitoleic and oleic acids in CU compared to HV. Importantly, the cumulative exposure to cannabis positively correlated with palmitic acid levels. We then measured a de novo lipogenesis index (palmitate/linoleate) and observed an increased in this index in CU. To further validate this result, we then performed carbon isotope ratio analysis, which further suggested that the increase in palmitic acid is due to lipogenesis from added sugars. Additionally, the levels of pyruvate and lactate were lower in the CU group. Pyruvate levels were negatively correlated with palmitic acid levels. A previous study reported that glucose and insulin levels are lower in CU suggesting that an altered metabolism in these subjects. Thus, we will complement our analyses by assessing levels of glucose and endocannabinoids. To the best of our knowledge, this is the first report of changes in fatty acid metabolism in CU and further studies will be necessary to evaluate the significance of these findings.

**Pharmacology of the Minor Cannabinoids in Cultured Cells and Rodents.** Robert Laprairie, University of Saskatchewan, Canada

The Cannabis sativa plant contains more than 120 cannabinoids. Comparatively little is known about the vast majority of plant-derived cannabinoids beyond Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Pre-clinical research regarding the cannabinoids has largely focused on the type 1 and 2 cannabinoid receptors (CB1R, CB2R), which remain the most-characterized cannabinoid receptors. Δ9-THC treatment is associated with pain-relieving, appetite-inducing, and anxiolytic effects via CB1R; and anti-inflammatory effects via CB2R. In our group, we have been studying the pharmacology of 10 cannabinoids and 6 terpenes present in cannabis at lower amounts than Δ9-THC or CBD. These compounds have been assessed alone and in combination with one another for their (1) affinity to CB1R and CB2R, (2) modulation of intracellular signalling cascades, and (3) effects on pain, anxiety, and movement in mice.Our findings demonstrate many less-abundant plant cannabinoids are partial agonists of CB1R and/or CB2R but that terpenes have little-to-no impact on cannabinoid pharmacology at these receptors. Among the cannabinoid tested, Δ9-THC appears to be unique in its engagement of beta-arrestin2, which may explain its intoxicating properties and undesirable side effects. These initial data represent a first step toward understanding the complex pharmacology of cannabis and may improve our harms reduction approach to cannabis as well as the development of novel cannabinoid-based therapeutics.

**Scientific, Regulatory, and Commercial Considerations for Products Derived from cannabis Sativa L.** Douglas MacKay, CV Sciences, USA

Cannabis sativa L is one of the oldest plants embraced by humans. Chinese farmers grew it to make rope and clothing more than 4,000 years ago. Today more there are more than 150 million cannabis users making it one of the world's most popular recreational drug products. In the U.S. cannabis has been used to develop an FDA approved prescription drug and medical and/or recreational products derived from cannabis are legal in many states.

The patchwork of state cannabis regulations resulted a range of products from simple dried flower to highly innovative and technical delivery systems for quantified levels of individual cannabinoids. In addition, The Agriculture Improvement Act of 2018 known as the "2018 Farm Bill" made removed 'hemp' defined as Cannabis sativa L. with a THC content of no more than 0.3% from the Controlled Substance Act. The Farm Bill created a new legal market for cannabinoid products derived from hemp in all 50 states.

Cannabis derived products are a rapidly evolving product category, however there are layers of legal and

regulatory considerations for product development. Cannabis products can be considered medical or recreational in some U.S. states, while Cannabis derived drugs can be developed for interstate commerce. Hemp derived cannabinoid products are legal in all 50 states, however products marketed as drugs, cosmetics, dietary supplements, or medical foods are under the authority of the FDA. To date, FDA has been exercising unofficial enforcement discretion when regulating hemp derived cannabinoid products. This has led to confusion amongst retailers and marketers of these products.
This talk will provide an overview of cannabis products being sold under different regulatory paradigms. The state of cannabis science will be discussed in context with how each regulatory framework shapes product premarket scientific investments, allowed raw material, finished product composition, labeling, and intended use.

## Clinical and Pre-clinical Studies of Polyunsaturated Fatty Acids (AOCS & ISSFAL Joint Session)

*Chairs RJ Scott Lacombe, Dell Pediatric Research Institute, Dell Medical School, University of Texas at Austin, USA; Martha Belury, The Ohio State University, USA*

**Associations of n-3 Fatty Acid Status During Early Pregnancy with Perinatal Depression at 12 Months Postpartum in Urban South Africa: The NuPED Study.** Ester Osuna, Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Switzerland
Background: Perinatal depression can negatively affect the health of the mother and her offspring, and is more prevalent in low- and middle-income countries. N-3 polyunsaturated fatty acids (PUFAs) may play a role in the etiology of depression.
Objective: We assessed red blood cell (RBC) fatty acids (FAs) at < 18 weeks' gestation, and determined associations of FA patterns, the omega-3 index and selected FAs with perinatal depression at < 18, 22 and 36 weeks' gestation, and at 6 and 12 months postpartum among women in urban South Africa.
Methods: As part of the Nutrition during Pregnancy and Early Development (NuPED) cohort of 250 pregnant women in Johannesburg, South Africa, we analysed RBC total phospholipid FA composition (% total FAs) and determined FA patterns using principal component analysis. We used the Edinburgh Postnatal Depression Scale (EPDS) to identify women at risk for depression (EPDS score ≥9).
Results: The prevalence of depression was 25%, 19%, and 17% at < 18, 22 and 36 weeks' gestation, and 26% and 22% at 6 and 12 months postpartum, respectively. We identified four RBC FA patterns: high saturated FA; low essential FA and high trans FA; high ALA, EPA, and n-6 GLA; and high n-3 PUFA. We found a negative association of the high n-3 PUFA pattern and EPA with EPDS scores at 12 months postpartum ($\beta$=–1.30±0.58, p=0.028 and $\beta$=–8.6±3.8, p=0.027, respectively). Women with higher scores for the high ALA, EPA, and n-6 GLA pattern had lower odds for depression at 12 months postpartum (OR=0.5[0.2–0.9], p=0.025). Also, women with a higher n-6/n-3 ratio and an omega-3 index < 4.5% had higher odds for depression at 12 months postpartum (OR=2.1[1.1–4.1], p=0.027 and OR=11.2[2.2–56.6], p=0.004, respectively).
Conclusion: Our results suggest that higher RBC n-3 PUFA status, especially EPA, during early pregnancy may lower the risk for postnatal depression at 12 months postpartum.

**Dietary Alpha-linolenic Acid Is Necessary for the Increase in Eicosapentaenoic Acid Following Docosahexaenoic Acid Intake.** Adam Metherel, Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Canada
It is well established that docosahexaenoic acid (DHA, 22:6n-3) intake results in elevated blood and tissue eicosapentaenoic acid (EPA, 20:5n-3). Recent evidence indicates that this increase is not due to increased retroconversion of DHA to EPA but is the result of a slowed metabolism and resultant EPA accumulation. The objective of this study was to determine whether the increase in EPA with DHA feeding is necessitated by the presence of alpha-linolenic acid (ALA, 18:3n-3) in the diet. Twenty-four 28-day old BALBc mice were placed on a 2% ALA diet for 4 weeks and then either 1) maintained on the 2% ALA diet, 2) switched to a 2% DHA diet or 3) switched to a 2% ALA + 2% DHA diet for 4 weeks. Tissues and blood were collected for determination of fatty acid concentrations, protein levels and enzyme activity. DHA levels were not different between the DHA and the ALA+DHA groups for plasma or liver, but were at least 59% and 109% higher compared to the ALA group in plasma (479 ± 42, nmol/mL ± SEM) and liver (7.5 ± 0.5, umol/g ± SEM), respectively. However, for both liver and plasma, EPA was 79–117% higher in the ALA +DHA group compared with both the ALA group and DHA group, with the latter two not different. Elongase 2 protein levels were not different between any dietary groups, however, elongase 2 enzyme activity was 26–31% lower in the DHA compared to ALA fed groups. Dietary ALA combined with DHA is necessary for the concomitant rise in blood and tissue EPA levels and DHA feeding appears to inhibit elongase 2 via a post-translational mechanism, and provides additional evidence that increases in EPA are the result of a slowed EPA metabolism.

**Elucidating the Effects of Dihomo-γ-linolenic Acid (DGLA) and the Dgla-rich Green Microalga in a Zebrafish Model of Gut Inflammation.** Ekaterina Novichkova, Weizmann Institute of Science

Polyunsaturated fatty acids are precursors for a great variety of lipid mediators, which are physiologically active substances involved in many functions, including inflammation processes. Dihomo-γ-linolenic (DGLA) acid is an omega-6 LC-PUFA. Studies have reported its anti-inflammatory and immuno-modulatory activities. DGLA is a precursor of prostaglandin E1 (PGE1), which initiates a cascade of anti-inflammatory events, and 15-(S)-hydroxy-8,11,13-eicosatrienoic acid, which is known for its anti-inflammatory properties.

Microalgae is a promising resource of LC-PUFA that play a pivotal role in human health. In our lab we developed the delta-5 desaturase mutant strain P127 of the arachidonic acid producing microalga Lobosphaera incisa, which represents the unique source of DGLA. The advantageous feature of this strain is the ability to accumulate high amounts of DGLA under nitrogen starvation conditions. The goal of this research was to reveal the potential of DGLA and other biological active components of P127 in ameliorating outcomes of gut inflammation. To this aim, we established a model of Inflammatory Bowel Disease (IBD) in zebrafish (Danio rerio). Biomass of P127, grown under nitrogen-replete and depleted conditions, and commercial DGLA was applied as a dietary supplement to Zebrafish for 4 weeks. Gut inflammation was induced by intubation with 120 μM trinitrobenzenesulfonic acid (TNBS), and gut samples were collected before and 24-h post-induction for gene expression analysis, histochemistry and microbiome sequencing. Histological examination indicated that diets supplemented with DGLA-rich algae biomass (after nitrogen starvation) significantly improved gut condition after TNBS challenge. We also observed a promoting effect of the diet enriched with non-starved algae on expression of several genes, involved in immune response, such as IL-10, Defensin, Lyzozyme and some others. Our findings are consistent with diversified functional properties of P127 biomass in zebrafish mucosal health, spanning from immunomodulation to reducing gut inflammation outcomes.

**High-oleic Ready-to-use Therapeutic Food with or Without Docosahexaenoic Acid Supports Normal Growth and Recovery in Malnourished Malawian Children: A Randomized Controlled Trial.** RJ Scott Lacombe, Dell Pediatric Research Institute, Dell Medical School, University of Texas at Austin, USA

Ready-to-use therapeutic foods (RUTF) are energy-dense, nutrient-enriched pastes widely prescribed for the treatment of uncomplicated severe acute malnutrition (SAM). Most common formulations of RUTF contain high levels of linoleic acid, minimal α-linolenic acid and zero long chain n-3 polyunsaturated fatty acids. As a result, standard RUTFs fail to maintain circulating levels docosahexaenoic acid (DHA) in vulnerable children recovering from SAM. The present randomized, quadruple-blind, controlled trial tested the efficacy of a high-oleic acid RUTFs with (HO+DHA-RUTF) and without DHA (HO-RUTF) against a standard RUTF (STND-RUTF) for the treatment of uncomplicated SAM in children under 5 years of age from rural Malawi. The primary outcome was the comparison of recovery from SAM over 12 weeks follow up. Secondary analysis investigated changes in growth indices and plasma phospholipid DHA levels (% of total fatty acids) at 4-weeks follow up. Among the 603 children enrolled, 64% (113/178) receiving STND-RUTF, 67% (135/202) receiving HO-RUTF, and 59% (131/223) receiving HO+DHA-RUTF recovered ($P = 0.22$). Median time to recover did not differ across treatment groups ($P = 0.17$). The rates of weight gain, change in mid-upper arm circumference, and length did not differ across treatment arms ($P = 0.47$, $P = 0.69$, and $P = 0.60$, respectively). After 4-weeks, plasma phospholipid DHA was highest in children receiving HO+DHA-RUTF (3.5%, 95% CI 3.34 to 3.65%) compared to children receiving either HO-RUTF (2.6%, 95% CI 2.5 to 2.8%) or STND-RUTF (2.7, 95% CI 2.5 to 2.9%) treatments ($P < 0.001$). Our results indicate that HO+DHA-RUTF and HO-RUTF formulations are as effective in the treatment of uncomplicated SAM in children under 5 years of age. Furthermore, compared to standard RUTF treatment, the DHA containing RUTF formulation yielded significantly higher circulating DHA levels, which may help to support neural development during an otherwise compromised developmental period. (ClinicalTrials.gov Identifier: NCT03094247)

**Modulation in Dietary Linoleic Acid Intake Results in Differential Transcriptomic Response to Ethanol Binge.** Chuck Chen, National Institute on Alcohol Abuse and Alcoholism, USA

Background: Substitution of dietary animal fats with vegetable oils has been shown to increased preference for ethanol. However, it remains unclear if alcohol use behaviour may be modulated differently by dietary n-6 and n-3 polyunsaturated fatty acids (PUFA). Objective: 1) Investigate striatal transcriptomic responses to ethanol exposure in mice fed diets varying in linoleic acid (LNA) and n-3 PUFA (EPA and DHA) and 2) examine if reduction of LNA and/or supplementation of n-3 PUFA will lower voluntary alcohol consumption. Methods: Behavioral cohort: Time-pregnant C57BL/6J dams were randomized to one of four custom dietary interventions varying in LNA and n-3 PUFA levels. Male offspring continued their respective maternal diet for 8 weeks before ethanol exposure. Mice were exposed to six cycles of 5-day 20%v/v ethanol for 4 hours in the dark and 2-day abstinence. RNA-Sequencing cohort: Same dietary interventions were applied. 15-week-old male offspring were subjected to a 9-day 2 g/kg and

1-day 5 g/kg ethanol gavage paradigm. Striatum was collected for next generation RNA sequencing after the last dose of gastric gavage. Results: Mice fed low LNA diets had significantly more differentially expressed genes in striatum after ethanol exposure as compared to mice fed high LNA diets. Pathway and network analyses found that mice fed low LNA diets and exposed to ethanol had transcriptomic changes associated with abnormal neuronal and synaptic structures as well as synaptic vesicle cycle and transmission. There was a significant 29% reduction in voluntary ethanol binge drinking in mice fed low LNA and low n-3 PUFA diet as compared to other dietary fatty acid interventions. Conclusion: Dietary fatty acids may alter striatal transcriptomic response to ethanol which may result in lowering of voluntary ethanol binge drinking, findings potentially applicable to a major public health concern.

## Lipid Oxidation Consequences to Health, Nutrition and Toxicity

*Chairs Jake Olson, TerViva Bioenergy Inc., USA; Karen Schaich, Rutgers University, USA; Teruo Miyazawa, Tohoku University*

**Are Lipid Oxidation Products Consumed in Foods Toxic? If So, Where**? Karen Schaich, Rutgers University, USA

As consumers become increasingly concerned with safety of their food supplies, questions have been raised concerning potential toxicity of lipid oxidation products consumed in mild to moderately rancid foods. A first look at this important issue was presented at AOCS in 2017. Inconsistent evidence and contradictory claims for pathological changes led to an extensive study of the literature to find reasons for the differences and develop a unified paradigm that could be used to guide food stabilization for safety. The present paper is the result of that study, providing details of the endogenous detoxification mechanisms that decompose dietary lipid oxidation products throughout the gastrointestinal tract and prevent their absorption, how serious problems in design of experiments with dietary excess and imbalance distort experimental results, and how other dietary components modify lipid effects. Changes observed following lipid feeding are considered in the context of normal physiology, metabolism, and detoxification, and ordinary expectable responses are differentiated from overt toxicity. Evidence is presented showing that uptake of lipid oxidation products and associated systemic effects are actually minimal due to detoxification, but damage to epithelial cells and microflora in the gastrointestinal tract before this detoxification can be extensive. Observations are integrated into a proposal for active involvement of dietary oxidized lipids and their co-oxidation products in gastrointestinal rather than systemic pathologies. Such action of lipid oxidation and co-oxidation products may contribute to current increases in inflammatory bowel diseases.

**Dietary Oxidized Fats Affect Gut Morphology, Microbial Ecology and Prepubertal Development.** Folagbayi Arowolo, University of California, San Francisco, USA; Kent Willis University of Alabama at Birmingham, USA; Ibrahim Karabayir, Loyola University Chicago, USA; Oguz Akbiligic, Loyola University Chicago, USA; Morgan Blaser, University of Wisconsin-Madison, USA; Xing Yang, University of Wisconsin-Madison, USA; Thong Phan, University of Wisconsin-Madison, USA; Clara Sato, University of Wisconsin-Madison, USA; Jeffrey Booth, University of Wisconsin-Madison, USA; Joseph Pierre, University of Tennessee Health Science Center, USA; Dhanansayan Shanmuganayagam, University of Wisconsin-Madison, USA

Westernized diets contain elevated levels of dietary fats and are associated with increased risk of obesity and other chronic diseases. These diets are increasingly consumed by children in the United States, potentially driving future risk of chronic disease. Additionally, cooking practices such as high heat frying and increased use of oxidizable sources of fats have introduced high levels of lipid oxidation products (LOPs) into the diet. The effects of these highly reactive compounds in the diet are largely unstudied, especially in the gut where these compounds exist in higher concentrations. Given that the gut microbiome can be influenced by dietary components and then in turn influence energy metabolism and body composition, we investigated the effects of consuming LOPs on growth, body composition, gut bacterial and fungal communities and gut morphology, using a prepubertal pig model. We observed that the presence of LOPs in the high fat diet reduced rate of body weight and body fat gain. The gut microbiome was altered by both high fat and the presence of LOPs, with notable changes in the abundances of Turicibacterales, Spriochaetales, RF39, Lactobacillales and Erysipelotrichales. The mycobiome was dominated by Kazachstania, a porcine specific yeast, which was only minimally influenced by the dietary regimen. Application of machine learning identified dietary fat and LOPs as strong predictors of body fat in the animal model. The abundance of the genus Methanobrevibacter was also an important predictor of body fat. Morphologically, LOPs intake also led to significant reductions in jejunal and ileal crypt depths. Our findings highlight the need for further studies on the biological effects of LOPs which have become ubiquitous in human, livestock and pet diets in developed countries.

**Dietary Oxidized Lipids Affect Phospholipid Profile and Fatty Acid Uptake in Gastrointestinal Cells.** Yifan Bao, Department of Physiological Chemistry,

Faculty of Chemistry, University of Vienna; Austria; Sarah Fruehwirth, University of Vienna, Austria; Immanuel Stricker, University of Vienna, Austria; Mohammed Salim, University of Vienna, Austria; Sofie Zehentner, University of Vienna, Austria; Veronika Somoza, Leibniz Institute for Food Systems Biology, Germany; Marc Pignitter, University of Vienna, Austria

Consumption of oxidized lipids in foods has been suggested to induce adverse health and toxic effects. However, reports about the structure-related activity of oxidized lipids are scarce and have mainly focused on the toxicity of highly reactive lipid aldehydes. We aimed at studying the impact of in vitro-digested oxidized lipids isolated from vegetable oils on the metabolic response of gastrointestinal cells.

Oxidized lipids from non-digested oils induced the formation of cellular phospholipids consisting of unsaturated fatty acids by about 40%–60% in human gastric tumor cells. The in vitro digested oils failed to affect the phospholipid metabolism. Hence, the gastric conditions inhibited the phospholipid-regulating effect of oxidized triacylglycerols, with potential implications in lipid absorption. An enhanced abundance of phosphatidylcholines could also be confirmed by incubating Caco-2 cells with the polar fraction of heated vegetable oils, 13-HODE and hexanal, whereas 13-HpODE and unheated oil did not affect the phospholipids. However, oxidized lipids-induced regulation of phospholipids did not seem to play a decisive role in lipid absorption since fatty acid uptake by Caco-2 cells was enhanced by 13-HpODE and decreased by hexanal.

**Lipid Oxidation and the Formation of Processing-induced Toxicants in Foods: Acrylamide and Heterocyclic Aromatic Amines.** Rosario Zamora, CSIC-Instituto de la Grasa, Spain

Consequences of lipid oxidation in health, nutrition, and toxicity are not only related to oxidized lipids themselves, but also to the ability of lipid oxidation products to generate processing-induced food toxicants. The objective of this paper is to discuss the role of lipid-derived reactive carbonyls to produce acrylamide and heterocyclic aromatic amines. Thus, the reaction conditions by which lipid oxidation products are able to produce these compounds as well as the mechanisms involved in their formation will be discussed. This discussion will also include the control of the formation of acrylamide and heterocyclic aromatic amines by carbonyl-scavengers. Obtained results show that lipid oxidation both plays a major role in the formation of these processing-induced food toxicants in fat-rich products and is the main starting point for the production of some of them.

**Lipid Oxidation and the Formation of Processing-induced Toxicants in Foods: Acrylamide and Heterocyclic Aromatic Amines.** Francisco J. Hidalgo, CSIC-Instituto de la Grasa, Spain

Consequences of lipid oxidation in health, nutrition, and toxicity are not only related to oxidized lipids themselves, but also to the ability of lipid oxidation products to generate processing-induced food toxicants. The objective of this paper is to discuss the role of lipid-derived reactive carbonyls to produce acrylamide and heterocyclic aromatic amines. Thus, the reaction conditions by which lipid oxidation products are able to produce these compounds as well as the mechanisms involved in their formation will be discussed. This discussion will also include the control of the formation of acrylamide and heterocyclic aromatic amines by carbonyl-scavengers. Obtained results show that lipid oxidation both plays a major role in the formation of these processing-induced food toxicants in fat-rich products and is the main starting point for the production of some of them.

**Recent Advances in the Toxicological Effects of Dietary Oxidized Amino Acids.** Mario Estevez, University of Extremadura, Spain; Alexis Arjona, Servicio Extremeño de Salud, Spain; Carmen Dueñas, Servicio Extremeño de Salud, Spain

The last couple of decades have witnessed an increasing interest in protein oxidation as a cause of food deterioration. Food proteins are oxidized during processing and storage, leading to the formation of cross-links and formation of oxidized amino acids. The impact of protein oxidation in foods includes texture changes, impaired protein functionality and loss of nutritional value. Moreover, recent studies have revealed that the intake of oxidized proteins and amino acids may contribute to in vivo oxidative stress and participate in the onset of pathological conditions. The present review aims to provide an overview of the role of oxidized proteins on food quality and safety and make emphasis on the molecular mechanisms behind the toxicological effects of lysine oxidation products, namely, allysine and amino adipic acid (AAA). Allysine is the most abundant protein carbonyl and is typically used as marker of protein oxidation in biological systems. Recent studies revealed that the acute intake of food-compatible amounts of allysine, leads to increased carbonylation of the gastrointestinal tract, plasma and internal organs of Wistar rats. Furthermore, the hepatoxicity of this species in the liver would explain impairment of glucose and lipid metabolism with increased circulating cholesterol and cholesterol oxides. AAA is known to be absorbed and display bioactive effects in the pancreas. Recent studies identified AAA as early marker of type II diabetes and obesity in children. Mechanistic studies in human and mice cultured cells have recently revealed the mechanisms by which this species is able to impair both exocrine and endocrine function of pancreas. A detailed proteomic study of AAA effects in CACO-2 cells shows the ability of this species to disturb mitochondrial redox status by acting as analog of glutamic

acid. These recent advances highlight the biological relevance of dietary protein oxidation and encourage scientists to perform further studies.

## Emerging Sources of Protein

*Chairs James House, University of Manitoba, Canada; Rotimi Aluko, University of Manitoba, Canada; Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada*

**Algae: A Key Protein Source for the Development of New Functional Ingredients.** Lucie Beaulieu, Université Laval, Canada

The increasing world population has driven the search for unconventional protein sources as ingredients to be incorporated in new high-value products. Algae can be viewed as a sustainable protein source for the future and a promising food ingredient due to their nutritional value, distinctive flavours and richness in bioactive compounds. Macroalgae from cold waters of eastern coast Canada have been prospected for discovering peptides having biological activities with potential health benefits. Antioxidants, antimicrobials, and inhibitors of the angiotensin-converting enzyme (ACE), an enzyme involved in blood pressure regulation, have been demonstrated. Several bioactive peptides have been observed following enzymatic hydrolysis of algal proteins. Significant differences in bioactivities of protein and peptide extracts of wild and cultivated macroalgae have been highlighted. Furthermore, edible macroalgae have been studied for their nutritional value and culinary potential according to their regional origin and time of harvesting, and to their processed (e.g., fresh vs bleaching, drying, freezing). Macroalgae have also been incorporated in different food models such as dairy products. This study confirmed the potential of macroalgae to be combined with fermented foods such as cheese, or yogurt, without causing a negative impact on the global evolution of the product. Their presence could improve the nutrient diversity and the nutritional quality by including algal proteins. These results can be ultimately viewed as a powerful way to drive the development of a tailored production and extraction of high value molecules. Our research activities will lead to new active algae-based food with beneficial health properties that can be seen as functional foods that will reach the growing healthcare and well-being market.

**Comparison, Optimization, and Identification of Bioactive Peptides Targeting Markers for Type Two Diabetes from Different Varieties of Chickpea.** Karla Acevedo-Martínez, University of Illinois, USA

Chickpea has gained relevance for its high global consumption, low allergenicity and bioactivity in prevention of chronic diseases. The objective was to compare five varieties of chickpea (Cicer arietinum), optimize the production of hydrolysates, identify peptides and determine biochemical markers for type-2 diabetes (T2D). Protein of ground raw, precooked and cooked chickpea was extracted at pH 9.0 and precipitated at pH 4.5. SDS-PAGE was used to compare protein profiles of the isolates. Simulated pepsin-pancreatin digestion, and bromelain were used to obtain hydrolysates. Response surface methodology was used to optimize dipeptidyl peptidase (DPPIV) inhibition, as a marker of T2D. Liquid chromatography mass spectrometry, BLAST and BIOPEP databases were used to identify and characterize peptides. Soluble protein was significantly different among varieties ($13.3 \pm 1.0$ to $19.1 \pm 1.9$ g/100 g). Protein fractions of higher molecular weight were comparable to 11S legumin (350 kDa) and 7S vicilin (180 kDa). Albumin fractions 2S (20 - 26 kDa) and vicilins were present in most samples after 2 h cooking. DPPIV IC50 for pepsin-pancreatin hydrolysates showed higher potency in raw than precooked or cooked chickpea (Billy bean: 0.17, Myles: 0.34, Sierra 2.21 and Nash 5.52 mg of protein/mL). Peptide sequences (KSEGGGPGGGGGAR, PPGGKG, PPGGGK, TPGGKGF) from parent trypsin inhibitors and legumin-like proteins and with DPPIV inhibitory capacity had high hydrophobicity (12.83–26.24 Kcal/mol) and pI (9.80–10.59). Optimization with bromelain hydrolysis showed a DPPIV inhibition of $95\% \pm 3$ for Sierra variety cooked for 15 min with 1:10 E/S ratio and hydrolysis time of 60 min. Peptides from bromelain hydrolysates with DPPIV inhibition were present in albumin fractions (EVLSEVSF) with 908.44 Da of molecular mass; and in legumin (VVFW, FDLPAL) with 549.29 and 674.36 Da, respectively. Results present conditions to optimize bioactivity and increase the value-added of chickpea to be used as functional ingredients.

**Conventional and Innovative Food Technologies for the Production of Edible Insect Ingredients.** Elvira Gonzalez de Mejia, University of Illinois, USA; Alain Doyen, Université Laval, Canada; Alexia Gravel, Université Laval, Canada

The consumer acceptability remains the main challenge to the integration of whole edible insects into Western food culture. However, the use of protein concentrate and isolate could decrease neophobia. Since the defatting of edible insects is the first step in producing these protein ingredients, it is necessary to compare the different defatting methods on lipid extraction and their impact on protein profiles and techno-functionalities.

In this study, the effects of six defatting methods were investigated on lipid extraction yield and fatty profiles of Tenebrio molitor meals as well as protein extraction and purification of defatted fractions. Moreover, the protein profiles of hexane-defatted and non-hexane-defatted

T. molitor meals and protein extracts were specifically studied. Finally, the effect of using hexane as defatting solvent was evaluated on protein solubility and foaming properties.

Results show that ethanol allowed a slightly higher lipid extraction yield (22.7–28.8 %) irrespective of the extraction method and insect meal. Palmitic acid (C16:0), vaccenic acid (C18:1 vaccenate) and linoleic acid (C18:2) were the three most abundant fatty acids identified in insect oils. The defatting method had low impact on protein extraction yield (~34%) and purification rates (~74%). Regarding more specifically hexane-defatted and non-hexane-defatted T. molitor meals, we showed that profiles for major proteins were similar between both samples. However, some specific differences (e.g., hexamerin 2) were observed. Protein solubility at pH 5, 7 and 9 was markedly lower for T. molitor meals (18 to 60%) compared to protein extracts (60 to 90%). A large increase in the foaming capacity, ranging from 546 and 629%, was observed for defatted fractions whereas foam stability decreased similarly.

In light of these results, the choice of defatting method represents an important parameter to consider in order generating edible insect protein ingredients with optimized techno-functional properties for specific food formulations.

**Effect of Solvent-de-oiling on the Protein Quality of Roller-milled Canaryseed Flour.** Suneru Perera, University of Saskatchewan / Keyleaf Life-Sciences, Canada; Dellaney Konieczny, University of Saskatchewan, Canada; Michael Nickerson, University of Saskatchewan, Canada

Canaryseed (Phalaris canariensis L.) is a ''novel'', true-cereal grain that contains comparatively high protein (~19–23%) and oil (~5–7%) content among other cereals. In 2015, it was approved by Health Canada and the FDA to have GRAS status. Although it has the potential to be developed as a source for protein ingredients for human consumption, the presence of high-oil content poses some technical challenges in producing low-oil-containing protein ingredients unless de-oiled. Since solvent-de-oiling is the most common method used in the industry, this study was carried out to understand the effect of solvent-de-oiling on the protein quality, i.e., techno-functional and nutritional properties, of roller-milled canaryseed flour (RMCF).

The dehulled canaryseeds were tempered to 13% moisture, roller milled, and de-oiled using hexane and ethanol to prepare flour. The isoelectric point of the control sample, i.e., non-de-oiled RMCF (N-RMCF), and ethanol-de-oiled RMCF (E-RMCF) were ~pH 4.6, while it was ~pH 5.5 for hexane-de-oiled RMCF (H-RMCF); however, the minimum solubility of all three flours (13.9–15.6%) was obtained at pH 5. The oil holding capacity (OHC) of N-RMCF and H-RMCF were not significantly different whereas, it was significantly higher in E-RMCF (OHC$=1.20 \pm 0.02$ g/g). On the other hand, the water holding capacities of de-oiled flours were significantly higher than that of the control and E-RMCF showed the highest value of $0.9 \pm 0.1$ g/g. DSC analysis of N-RMCF showed a protein denaturation peak at 95.2°C; in spite of de-oiling, both H-RMCF and E-RCMF showed protein denaturation peaks at 98.9°C. In terms of nutritional quality, a significant increment of invitro-PDCASS of de-oiled RMCF was observed than that of the control. The highest in vitro-PDCASS value ($28.8 \pm 0.1$) was observed in the E-RMCF sample. Overall, solvent-de-oiling did not show any evidence for posing a negative impact on the protein quality of RMCF.

**in vitro antioxidant and Antihypertensive Properties of Edible Cricket (Brachytrupes Membranaceus) Protein Derived Membrane Peptide Fractions.** Rotimi Aluko, University of Manitoba, Canada; J.A. Gborigyo Tsav-wua, College of Education, Nigeria; Fatima Abdulsalam, Federal University of Agriculture, Makurdi, Nigeria; Abraham Girgih, University of Agriculture, Nigeria

The objective of this study was to determine the in vitro antioxidant and antihypertensive properties of edible cricket protein meal (CRIPM), cricket protein hydrolysate (CRIPH) and its peptide fractions. CRIPM was digested sequentially using pepsin and pancreatin enzymes to mimic gastrointestinal digestion in human beings. The resultant CRIPH was then separated by membrane ultrafiltration into peptide fractions with sizes of < 1, 1–3, 3–5 and 5–10 kDa. The protein content of CRIPM (61.7%) was significantly ($P < 0.05$) lower than that of CRIPH (79.9%) and the peptide fractions (78.8–83.0%). The fractions showed better DPPH (81–85%), Superoxide (80–84%) and Hydroxyl (15–45%) radicals scavenging activities than glutathione (GSH) with lower activities of 66, 58 and 12%, respectively. GSH had the highest IC50 value of 0.45 mg/mL compared to the other samples IC50 value range of 0.34–0.41 mg/mL. CRIPM, CRIPH and their peptides chelated metals (31–69%) and reduced ferric ions (38–83%) compared to GSH (37 & 61%) to inhibit free radical destructive activities. Membrane fractionation resulted in peptides with excellent ACE and moderate renin inhibitory activities (77–9 & 58–7%, respectively). Molecular weight distribution of CRIPM and CRIPH showed that the samples were predominantly composed of LMW peptides (0.03–0.81 kDa) that could be responsible for the observed bioactivities. The study suggests that CRIPM, CRIPH and peptide fractions are potential therapeutic ingredients for the development of functional foods and nutraceuticals for the prevent and amelioration of oxidative stress and hypertension. Results could promote increased farming of edible crickets, thus providing economic benefits to all stake holders.

**Study of Linseed Protein Extraction and Purification: Impact of Process Conditions on Protein Functional and Structural Properties.** Sara Albe Slabi, LRGP-UMR CNRS 7274, France; Sophie Beaubier, LRGP-UMR CNRS 7274, France; Mbalo Ndiaye, Avril Group, France; Olivier Galet, Avril Group, France; Romain Kapel, LRGP-UMR CNRS 7274, France

Global demand for production of protein isolates from alternative plant sources is constantly increasing. Linseed proteins present excellent nutritional and functional properties making them attractive for various food applications. To date, few studies have focused on this particular protein source.

The goal of this research was to investigate the impact of extraction and purification conditions on both process performances and quality of final protein product. First, the effect of pH (6–8), NaCl concentration (0–0.5 M) and temperature (20–50 °C) on extraction performances including protein yield, concentration and purity, and total carbohydrate content in the extract was investigated using design of experiments. Then, the influence of five selected extraction conditions and pH (3–6) used for acidic precipitation on total protein yield was evaluated. Finally, functional and structural properties were compared for linseed protein isolates produced by two different process conditions.

As a result, the obtained models confirmed the synergic, positive impact of pH and NaCl concentration on linseed protein extraction yield. On the other hand, the high temperature leaded to the increase of carbohydrate extractability that decreased the protein concentration and purity. Purification yield depended on the extraction conditions (particularly NaCl concentration) and pH of acidic precipitation. Maximum total protein yields were achieved by extraction at pH 7.5 without NaCl following by protein precipitation at pH 4 and 5. Interestingly, linseed protein isolates produced under these two process conditions differed significantly in their functional (solubility, foaming, emulsifying) and structural properties.

## Protein Biofunctions

*Chairs Kaustav Majumder, University of Nebraska-Lincoln, USA; Apollinaire Tsopmo, Carleton University, Canada; Hitomi Kumagai, Nihon University, Japan*

**An Overview of Peptide and Protein Absorption.** Toshiro Matsui, Kyushu University, Japan

It still remains unclear whether bioactive small and oligopeptides are absorbed into the body in their intact form. Absorption of dietary bioactive proteins such as soy and maize is also questionable, although the intake of such proteins may evidentially exert health benefits such as anti-lipidemic and anti-obesity effects. At the intestinal transport systems di-/tripeptides are absorbed mainly via the carrier mediated-peptide transporter 1 (PepT1), and tetra-/pentapeptides are able to penetrate presumably via the tight junction. The limiting factors for absorption of peptides are not only the size, but also the resistance against degradation by proteases (and aging of animals), along with the affinity with transporters. DGYMP, an anti-muscle atrophy Cblin pentapeptide, DGYMP, could also be absorbed in rats in pentapeptide form, although a proportion was metabolized and detected as peptide fragments. Aging of spontaneously hypertensive rats (SHRs) promoted the absorption of dipeptides, whereas the absorption of oligopeptides was not affected by aging. These findings would be explained by enhanced expression of PepT1 and no enhanced expression of tight junction-related proteins in intestinal membrane of aged SHRs, compared to young. In this symposium we also introduce our finding that the oral intake of the reportedly bioactive ß-conglycinin, a soybean protein, to rats could cause the production of oligopeptides in rat bloodstream.

**Anti-inflammatory Effect of Kokumi-active Dry-edible Beans-derived Dietary γglutamyl Dipeptide (γ-Glu-Val).** Kaustav Majumder, University of Nebraska-Lincoln, USA

γ-glutamyl peptides are naturally occurring dietary peptides where the peptide bond is formed through the γ-carbon of glutamic acid. These peptides exert Kokumi flavor through the activation of Calcium Sensing Receptors (CaSR) and are also known as Kokumi peptides. Dietary γ-glutamyl peptide (γ-Glu-Val: γ-EV) can reduce gastrointestinal inflammation in a mouse model of inflammatory bowel disease (IBD) and sepsis via allosteric activation of CaSR, a G protein-coupled receptor present in various tissues, essential for calcium homeostasis in the human body. In vascular endothelial cells, CaSR regulates blood pressure via activation of intermediate conductance Ca2+-sensitive potassium channels. Given the prevalence of vascular diseases, the present study aims to evaluate the therapeutic potential of γ-EV in modulating vascular inflammation. The pre-treatment of γ-EV (1mM for 2h) can significantly reduce the tumor necrosis factor-α (TNF-α)-induced vascular inflammation in human aortic endothelial cells (HAECs), by lowering the expression of inflammatory adhesion molecules (VCAM-1 and E-Selectin), chemokine (MCP-1), and cytokines (IL-6 and IL-8). The signaling modulators, such as β-arrestin2, CREB, and p65, play a crucial role in the CaSR-dependent anti-inflammatory effect of γ-EV in HAECs. Due to its unique structure, γ-EV is resistant to gastric digestion and can be absorbed by the intestinal epithelial cells via paracellular as well as PepT1 transporter. Furthermore, the study develops a scalable extraction

method to extract the naturally occurring γ-glutamyl peptides from Nebraskan Great Northern Beans (GNB). The extract exhibits a similar anti-inflammatory effect at a dosage of 50mg/mL in human endothelial cells. γ-EV is the most abundant (53 ng/mg) γ-peptide identified in the GNB extract, indicating the possible role of γ-EV in exhibiting the anti-inflammatory activity. Thus, the present study suggests the potential use of dry-edible beans in the development of functional foods for the prevention and management of vascular inflammatory diseases such as atherosclerosis.

**Bioprocessing: Effects on Biological Activities of Proteins, Hydrolyzed Proteins, and Peptides.** Apollinaire Tsopmo, Carleton University, Canada

Proteins are sources of nutrients and possess functional properties important to many foods. Intact proteins and their derived peptides are also biologically active with properties such antioxidant, anti-microbial, reduction of the risks of developing chronic diseases through various mechanisms. Those activities are affected by the food matrix and the bioprocessing method. There are procedures that focus on the enhancement of proteins extraction yields or functions through the removal of polysaccharides or antinutrients such as phytates. There are other related to the use proteases to modify the digestibility and fermentability of proteins, and to release bioactive active peptides.

Data from our laboratory have showed that using polysaccharide degrading enzymes the extraction of proteins from cereal brans was enhanced by up to 1.7-fold. There was also an increase in protein content from 54% in the control to 70% when the hydrolyzing polysaccharide enzyme was Viscozyme at 3 units/g bran. Higher extraction yields and protein contents translated in 3-fold increase of the preroxyl scavenging activity upon proteolytic hydrolysis of the proteins. In another work, we found that bioprocessing of pulses using germination and solid-state fermentation protein digestibility was increased by up to 3-folds. The samples inhibited dipeptidyl peptidase-4 and α-glucosidase to various degree dependent on the bioprocessing conditions.

**Digestive Survival of Milk Proteins and Release of Antimicrobial and Immunomodulatory Milk Peptides.** David, Dallas, Oregon State University, USA

Justification: Milk proteins have evolved to benefit the suckling neonate. The extent to which most of these proteins survive within the infant (for human milk) and adult (for bovine milk) remains mostly unknown, and thus their bioactive potential is unclear. For many milk proteins, partial digestion releases fragments—peptides—with known antimicrobial, prebiotic, immune-modulating, calcium-delivery, antihypertensive, and pain-modulating activities. The extent to which these peptides survive within the digestive tract need to be further examined to determine their biological relevance.

Objective: Determine the survival of milk proteins and release of bioactive peptides in the stomach and intestine of human infants (human milk) and adults (bovine milk).

Methods: Milk and infant/adult digestive samples were analyzed using mass spectrometry-based peptidomics, protease assays, ELISA and bioactive peptide database searching to determine how proteins are degraded within the digestive system and which potential bioactive peptides are released.

Results: Milk protein degradation begins within the mammary gland with milk proteases, releasing thousands of peptides, many of which are bioactive. In the stomach and intestine of human infants, both infant-produced and milk-derived proteases actively degrade milk proteins, releasing thousands of new peptides while allowing the survival of some milk proteins. In adults, we demonstrated that a key bioactive ingredient from bovine milk, kappa-casein glycomacropeptide does not survive to the intestine.

Significance: Peptidomic, proteomic and enzyme analyses enable precise characterization of protein digestion and bioactive peptide release in infants (human milk) and adults (bovine milk). Our ongoing research is characterizing which peptides are antimicrobial and immunomodulatory with in vitro assays and preparative liquid chromatography-based peptide fractionation.

**Evaluation of Allergenicity of Deamidated and/or Hydrolyzed Wheat Gliadin by Cutaneous Sensitization.** Hitomi Kumagai, Nihon University, Japan; Ryosuke Abe, Nihon University, Japan; Narumi Matsukaze, Nihon University, Japan; Yusuke Yamaguchi, Nihon, University, Japan; Hitoshi Kumagai, Kyoritsu Women's University, Japan

As protein hydrolysis often increase surface activities such as foaming and emulsifying properties, the hydrolysates are sometimes used in foods, cosmetics and shampoos. However, hydrochloric acid (HCl)-treated wheat protein (HWP) added in facial soap was reported to induce immediate hypersensitivity when wheat products are taken after using the soap for a certain period of time. Proteins are not only hydrolyzed but also deamidated by HCl treatment. Therefore, the present study aimed to examine which structural change is responsible for the induction of cutaneous sensitization by comparing the allergenicity of deamidated and/or peptide-bond-hydrolyzed wheat gliadin. After deamidated-only, hydrolyzed-only, and deamidated and hydrolyzed gliadins were cutaneously administered to mice for several weeks, the corresponding gliadin was intraperitoneally injected and allergenicity was evaluated. Deamidated and hydrolyzed gliadin induced severe allergic reaction by cutaneous administration, while deamidated-only and hydrolyzed-only gliadin barely showed allergic response. This result suggests that both deamidation and peptide-bond

hydrolysis are necessary to induce immune hypersensitivity by cutaneous administration.

**Novel Insights into the Transport Mechanisms of Bioactive Peptides from the Mucus Perspective.** Xiaohong Sun, Qiqihar University, China (People's Republic); Raliat Abioye, University of Ottawa, Canada; Ogadimma Okagu, University of Ottawa, Canada; Chibuike Udenigwe, University of Ottawa, Canada

Justification: The mucus layers act as protective barriers by covering the intestinal epithelium. However, it is not well defined how bioactive peptides interact with and penetrate through the mucus layers prior to reaching the underlying epithelium. Thus, current understanding of the transport mechanisms of bioactive peptides is not comprehensive.

Objective: The objectives of this study were to evaluate the mucin-binding and mucin-permeation capacities of bioactive peptides, and to understand the relationship between mucin interaction and the peptide structure.

Methods: Bioactive peptides with different physiochemical properties were selected. Mucin-binding activity of peptides was evaluated by a novel model that was established based on the specific interaction between lectin and mucin. The potential binding sites between peptides and mucin were determined by the fluorescence quenching assay. The effect of peptide binding on mucin morphology was imaged using fluorescence microscopy. Mucin-permeation property was determined by a biosimilar porcine mucus in vitro model.

Results: Among 7 tested peptides, KIPAVF and KMPV showed the strongest binding activity to soluble mucin and whole mucin, respectively. YMSV exhibited the lowest binding activity to both soluble and whole mucins. Fluorescence quenching assay indicated that the peptides likely bound to the glycan moieties of mucins. Fluorescence imaging demonstrated mucin exhibited distinct morphologies when it interacted with the different peptides. The mucus permeability of peptides was negatively correlated with their mucin-binding activity. Specifically, 13% of the peptide YMSV (with the lowest mucin-binding activity) permeated through the mucus layer, while only 3% of peptide KMPV (with the highest mucin-binding activity) passed through the mucus after 3 hours.

Significance: This work provided novel insights into the transport mechanisms of bioactive peptides, which is indispensable in understanding peptide absorption and bioavailability.

## Health and Nutrition Poster Session

*Hongbing Fan, University of Alberta, Canada*

**Cecal microbial community in laying hens influenced by type and dosage of dietary n-3 polyunsaturated fatty acids.** Mohamed Neijat, University of Manitoba, Canada; Jemaneh Habtewold, Agriculture and Agri-Food Canada, Canada; Shengnan Li, University of Manitoba, Canada; Mingyan Jing, University of Manitoba, Canada; James House, University of Manitoba, Canada

This study evaluated the effects of type and dosage of n-3 polyunsaturated fatty acids (PUFA) on the composition of cecal microbial communities of laying hens. Lohmann LSL-Classic hens (n=40, 10 per treatment) were randomly assigned to one of 4 corn-soybean-based diets containing either flaxseed oil (FO) or marine algae (MA), each type supplying doses of 0.20 and 0.60% of total n-3 PUFA in the diet. At the end of week 8, liver and cecal digesta samples were obtained for fatty acids and microbial analyses, respectively. The V3-V4 hypervariable region of 16S rRNA genes was sequenced using Illumina Miseq® platform. A distinct clustering pattern (P=0.002) of bacterial community was noted, particularly for MA-fed group. The relative abundances of predominant phyla (Firmicutes and Bacteroidetes) were not significantly different between FO- vs. MA-fed groups, however, less abundant phylum, Tenericutes, were significantly (P< 0.0001) greater in the FO- than MA-fed groups. The latter is often associated with immunomodulation. Although the relative abundance of Bacteroides was enriched (P< 0.05) at a lower dosage of FO (0.20%) compared to other treatments groups, the abundance of this genus at higher dosages (0.60%) of FO and MA was negatively correlated with n-3 PUFA content in the liver. In addition, a higher dose of FO (0.60%) and both dosages of MA supplementation enhanced (P< 0.05) the abundance of bacteria belonging to Firmicutes, including Clostridium and Ruminococcus. Moreover, the latter genera as well as Lachnospiraceae were positively correlated (P< 0.05) with total n-3 PUFA content in the liver with higher dosages of both FO and MA diets. In summary, the selective enrichment of specific microbes in the ceca of hens highlights the potential role of dietary manipulation of type and dosage of n-3 PUFA towards regulating the enrichment levels of n-3 PUFA in tissues and possibly immune homeostasis in birds.

**Effect of 6,9,12,15-Hexadecatetraenoic Acid (C16:4n-1)-Ethyl Ester on Lipid Contents and Fatty Acid Compositions in Blood and Organs of Mice.** Ryota Hosomi, Kansai University, Japan; Toshifumi Tanizaki, Bizen Chemical Co., Ltd., Japan; Shintaro Ikawa, Kansai University, Japan; Tadahiro Tsushima, Bizen Chemical Co., Ltd., Japan; Yoshihisa Misawa, Bizen Chemical Co., Ltd., USA; Naomichi Baba, Bizen Chemical Co., Ltd., Japan; Munehiro Yoshida, Kansai University, Japan; Kenji Fukunaga, Kansai University, Japan

The 6,9,12,15-hexadecatetraenoic acid (C16:4n-1; HDTA) is a polyunsaturated fatty acid with a double bond at the carbon at the terminal methyl group of the

fatty acid molecule. HDTA is present in small amounts in lipids such as fish oil. However, the metabolic and biological regulatory mechanisms of dietary HDTA is not clear. Therefore, the aim of this research was to investigate the intake of HDTA-ethyl ester (EE) on the lipid contents and fatty acid compositions in serum and organs of mice. Male C57BL/6J mice (4-weeks-old) were fed a 7% fat diet containing corn oil (CO), HDTA-EE, or eicosapentaenoic acid (EPA)-EE. HDTA-EE and EPA-EE were added to the diets so that the fatty acid composition of HDTA and EPA in the diets were 10 mol %, respectively. After the mice had been fed the experimental diets for 28 days, we analyzed the lipid contents and fatty acid compositions in serum, liver, white adipose tissue (WAT), and brain. The serum triacylglycerol (TAG) content in the HDTA-fed mice were lower compared with the CO- and EPA-fed mice, whereas the serum cholesterol content in the HDTA-fed mice did not change compared with the CO-fed mice. As a result of fatty acid composition analysis, HDTA was only detected in WAT but not in serum, liver, or brain. Interestingly, C18:4n-1, which is thought to be a metabolite of HDTA, was detected in serum, liver, and WAT. The present study demonstrated that the intake of HDTA has beneficial function of lowering serum TAG content.

**Effects of Dietary Phospholipids Prepared from the Japanese Giant Scallop (Patinopecten yessoensis) on Cholesterol Metabolism in C57BL/6J Mice.** Koki Sugimoto, Kansai University, Japan; Taiki Shinagawa, Kansai Univ, Japan; Ryota Hosomi, Kansai University, Japan; Munehiro Yoshida, Kansai University, Japan; Kenji Fukunaga, Kansai University, Japan

Our previous study showed that dietary scallop oil (SCO), containing high eicosapentaenoic acid and phospholipids (PL), decreased the serum and liver cholesterol contents in mice. In the present study, we focused on PL in SCO (SCO-PL) and investigated the mechanism of its cholesterol-lowering effect using mice. Five-week-old male C57BL/6J mice were fed lard based high-fat and cholesterol diets containing 3% soybean oil (SOY-TG group), or soybean PL (SOY-PL group), or triglyceride (TG)-type oil with almost the same fatty acid composition as SCO-PL (SCO-TG group), or SCO-PL (SCO-PL group) for four weeks. SCO-PL diet significantly decreased the serum and liver cholesterol contents compared with SOY-TG diet. The serum and liver cholesterol-lowering effect of SCO-PL was partly mediated by the enhancements of the liver Cyp7a1, which is a rate-limiting enzyme for bile acid synthesis, expression level. On the other hand, the ileum farnesoid X receptor (Fxr) and fibroblast growth factor 15 (Fgf15) expression levels in the SCO-PL group were significantly decreased compared with SOY-TG group. Additionally, the liver β-muricholic acid (MCA) content and Cyp2c70, which synthesize chenodeoxycholic acid to MCA, expression level in mice fed SCO-PL were significantly increased compared to mice fed SOY-TG. From these results, the increase of the liver Cyp7a1 expression level by dietary SCO-PL in partly through the reduction of the ileum Fgf15 expression level due to the increase of ileum taurine-conjugated βMCA, which is an antagonist of Fxr. This study indicated that dietary SCO-PL can be a health-promoting component for reducing cholesterol content.

**Efficacy of dietary γ-glutamyl peptide in modulating vascular inflammation.** Snigdha, Guha, University of Nebraska, Lincoln, USA; Kaustav Majumder, University of Nebraska-Lincoln, USA

Cardiovascular diseases (CVDs) accounts for around one-third of annual global deaths. Increased level of vascular inflammation contributes to the pathogenesis of various CVDs such as atherosclerosis. Dietary bioactive peptides have been shown to exhibit anti-inflammatory properties and could be used for the prevention or management of CVDs. Dietary γ-glutamyl valine (γ-EV), commonly found in dry- edible beans, reduced gastrointestinal inflammation via allosteric activation of Calcium-Sensing Receptors (CaSR). Thus, it was hypothesized that the intervention γ-EV might modulate the inflammatory responses in vascular endothelial cells via the activation of vascular CaSR. Human tumor necrosis factor-α (TNF-α) was used to induce inflammation in pre-treated (2h with γ-EV at 0.01mM, 0.1mM, and 1mM) human aortic endothelial cells (HAECs). TNF-α treatment significantly increased the expression of inflammatory adhesion molecules (VCAM-1, E-Selectin), chemokine (MCP-1), and cytokines (IL-6 and IL-8). However, the expression of these biomarkers was significantly downregulated by 1mM γ-EV pretreatment. Additionally, the anti-inflammatory effect of γ-EV was attenuated entirely by the intervention of selective CaSR inhibitor, NPS2143. Further investigation also led to the identification of certain key modulators, such as β-arrestin2, CREB, and p65, which were involved in the CaSR-dependent anti-inflammatory effect of γ-EV. The transport efficiency of γ-EV was evaluated through the monolayer of intestinal epithelial cells (Caco-2), and the apparent permeability (Papp) of the peptide was found to be 1.56x10–6 cm/sec. The mechanism of transport of the peptide across the Caco-2 cells was found to be both paracellular as well as PepT1-mediated. Thus, the study concluded that dietary γ-EV could significantly reduce vascular inflammation and can be used to develop functional foods or nutraceuticals for the prevention and management of CVDs.

**Emerging Metabolic Health Benefits of Palmitoleic Acid: From Bench to Human.** Gretchen Vannice, Wiley Companies, USA

Interest in identifying the metabolic and regulatory attributes of palmitoleic acid (cis 16:1 n-7) in human health

is rapidly increasing. A monounsaturated fatty acid, dietary sources of palmitoleic acid (POA) are most notably macadamia nuts and fish and it is available as a dietary supplement. Plasma levels are influenced by dietary intake, about 1–2 grams per day, and hepatic synthesis from palmitic acid (16:0) via SCD-1 activity. Indeed, POA has been described as a powerful blocker of SCD-1 activity and expression.

Found in circulation both free and in complex lipids, highest concentrations of POA occur in the liver and adipose tissue. Sex and regional adipose tissue (AT) differences have been reported, with higher POA levels in females, and in gluteal vs abdominal AT.

Cell culture and animal model work suggest a myriad of metabolic benefits of POA, ranging from enhanced glucose uptake, insulin sensitivity, appetite control (satiety), skin hydration, and immunomodulation to reductions in atherosclerotic plaque, inflammatory markers, and fatty liver.

Diet (macadamia nuts) and animal feeding studies report beneficial effects of POA on blood lipids. In population studies, circulating levels are associated with both increased and decreased risk of CVD and type 2 DM while pilot trial data reports significant reductions in LDL-cholesterol, triglycerides, and C-reactive protein. Two clinical trials investigating the role of POA are currently underway.

In sum, results of pre-clinical and pilot clinical work have been favorable and there is need for mechanistic and human research. This poster will summarize the state of the science and identify where further research is warranted.

**Enhanced oral absorption of vitamin E tocotrienols with nanoencapsulation: proof of concept in rat model.** Ju-Yen Fu, Malaysian Palm Oil Board, Malaysia; Doryn Meam-Yee Tan, Monash University, Malaysia; Puvaneswari Meganathan, Malaysian Palm Oil Board, Malaysia

Vitamin E tocotrienols are a group of compound found to have potent anti-oxidant and anti-inflammatory effect in the combat against chronic diseases. As a nutraceutical agent, more than 100 clinical studies have reported the health benefits of tocotrienols, specifically in the regulation of liver and cognitive function. One limitation of tocotrienols is their low bioavailability. This study describes the development of tocotrienol-loaded long circulating nanovesicles, aiming to enhance the absorption of tocotrienols after oral administration. Nanovesicles comprising of ascorbyl palmitate and hydrophilic polymer were prepared using film hydration method and stored in freeze-dried form. Female rats were randomized to receive tocotrienol-nanovesicles (Nano-T3) diluted in normal saline and unformulated tocotrienols diluted in vegetable oil (T3) via oral gavage at 5 mg/kg body weight. Blood samples were collected at pre-determined time points and quantified for α-, γ-, δ-tocotrienol levels. Nano-T3 measured the size of 176 ± 7 nm with polydispersity index of 0.23± 0.01 and zeta-potential of –60 ± 1 mV. They remained stable at storage for 2 months with less than 15% tocotrienol leakage. Three tocotrienol homologues (α, γ, δ) were quantified using validated HPLC system. Rats receiving T3 showed a gradual increase in tocotrienol plasma concentration from 0 to 4 hours while rapid elevation was observed in group receiving Nano-T3. Plasma concentration over time profile was significantly higher for γ- and δ-tocotrienol in NanoT3 group (p-value = 0.016 and 0.030 respectively). For γ-tocotrienol, maximum plasma concentrations (Cmax) and area under the curve (AUC0–24) were both increased by 8-fold with Nano-T3. For δ-tocotrienol, Cmax and AUC0–24 were enhanced by 17 and 13-fold respectively in Nano-T3 group. Nanovesicles are promising delivery system for tocotrienols with enhanced oral absorption of 8-fold and 17-fold in γ- and δ-tocotrienol respectively. Their application in food and nutraceutical product development is warranted.

**Linoleic acid (18:2n-6) to α-linolenic acid (18:3n-3) ratio modifies polyunsaturated fatty acid biosynthesis in human CD3+ T cells.** Johanna Von Gerichten, University of Surrey, United Kingdom; Annette West, University of Southampton, United Kingdom; Nicola Irvine, University of Southampton, United; Kingdom; Elizabeth Miles, University of Southampton, United Kingdom; Karen Lillycrop, University of Southampton, United Kingdom; Philip Calder, University of Southampton, United Kingdom; Barbara Fielding, University of Surrey, United Kingdom; Graham Burdge, University of Southampton, United Kingdom

Our previous work has found that immune cell activation and proliferation is partly determined by endogenous polyunsaturated fattyacid (PUFA) synthesis. This synthesis of n6- and n3-PUFA is regulated by a set of elongases and desaturases especially ELOVL5 andFADS1/2. However, the impact of the 18:2n6 to 18:3n3 ratio on PUFA synthesis in T cells is unknown.

Using peripheral blood CD3+ T cells from healthy volunteers (18–30 years; n=10), this study investigated the effect on PUFAsynthesis by differing 18:2n6:18:3n3 ratios, with a 10:1 or 4:1 ratio representing variations in typical dietary intake. T cells werecultured with or without concanavalin A (10 μg/ml) in 10% (v/v) pooled donor plasma with 18:2n-6 and 18:3n-3 (1:10labelled/unlabelled) for 48 hours. Total fatty acid composition of T cells was determined by gas chromatography (GC)-flame ionisation detection. PUFA synthesis wasdetected with GC-isotope ratio mass spectrometry using [d5]-18:2n-6 and [13C]-18:3n-3. Data were analysed by 2-way ANOVA.

The 4:1 ratio of 18:2n-6:18:3n-3 significantly lowered the n6/n3-PUFA ratio in activated T cells ([10:1] 8.0±1.1 vs. [4:1] 6.6±1.1, p=0.040). Furthermore, 4:1 ratio of 18:2n6:18:3n-3 resulted in a significantly higher concentration of [13C]-20:3n-3, the elongationproduct

of [13C]-18:3n-3 ([10:1] 0.03±0.02 vs. [4:1] 0.29±0.23 pmol/106 cells, p=0.034). Simultaneously, a significantly lowerconcentration of [d5]-20:2n-6, the elongation product of [d5]-18:2n-6 was observed ([10:1] 0.61±0.28 vs. [4:1] 0.09±0.07 pmol/106cells, p=0.0006). There were no significant changes detected in downstream synthesised PUFAs.
Lowering the ratio of 18:2n-6:18:3n-3, especially by using a higher 18:3n-3 concentration, shifts the elongation of de-novosynthesised n6-PUFA to n3-PUFA in activated T cells. These findings show for the first time that PUFA biosynthesis in T cells is modulated by the ratio of the essential fatty acid (EFA) substrates. This has implications for understanding the impact of dietary EFAson immune function.

**Nutritional and Phytochemical Analysis of Different Colored Taro Varieties in Hawaii.** Kento Senga, University of Hawaii at Manoa, USA; Kacie Ho, University of Hawaii at Manoa, USA; Jon-Paul Bingham, University of Hawaii at Manoa, USA
Taro is a staple crop that is widely consumed in Hawaii and parts of Asia. In Hawaii, commercial production is dominated by a single taro variety as a result of its high production yield. Different colored varieties, exclusive to the Hawaiian Islands, are known to be rich sources of bioactive compounds but their nutritional and phytochemical profiles are not well reported. As such, the purpose of this study was to analyze nutrient and phytochemical (polyphenol and carotenoid), content found within three different colored Hawaiian taro varieties: Maui Lehua (Purple), Piialii (Red), and Mana Ulu (Orange). Total phenolics and anthocyanins were estimated using the Folin–Ciocalteu assay and pH differential method, respectively. Carotenoids were quantified using high-performance liquid chromatography. Results indicated a difference in composition across varieties. Maui Lehua and Piialii cultivars showed higher phenolic and anthocyanin amounts when compared to the Mana Ulu cultivar. Thermal processing, on average, resulted in significant losses of phenolic and anthocyanin content across all taro varieties with Maui Lehua from 25.9 GAE μg /g DW to 6.95 GAE μg /g DW, Mana Ulu from 21.1 GAE μg /g DW to 6.22 GAE μg /g DW, and Piialii from 21.7 GAE μg /g DW to 11.0 GAE μg /g DW. Overall, the findings indicate that different colored taro varieties contain unique phytochemical profiles compared to the variety primarily used for commercial production. This research would support a more diverse agriculture industry by demonstrating potential nutritional benefits of alternative taro varieties and can be applied to better understand the composition of other agricultural products and our food supply.

**Nutritional values of infant formulas and flours and their adequacy with the requirements for infant aged from 6 to 12 months.** Mathilde Cancalon, CIRAD, France
Neonatal and infancy period is characterized by significant growth and development. From a nutritional point of view, this translates into specific requirements, notably in long chain polyunsaturated fatty acid (LC-PUFA) such as docosahexaenoic acid. To ensure an adequate nutritional intake different types of products, such as infant formulas and flours are designed. The aim of the present work is to assess the adequacy of these products with the infants aged from 6 to 12 months' requirements and with recent changes in European regulations. To reach this objective, a meta-analysis of the nutritional values of 91 follow-on formulas and 68 infant flours from northern et southern hemispheres was carried out with special focus on lipid profiles. For follow-on formulas, a significant homogeneity of macro- and micronutrient contents is observed from one product to another, and a good similarity of macronutrients contents with mature breast milk despite the persistence of differences in the lipids organisation and FA profiles. In the case of infant flours, the regulatory framework being less strict, an important heterogeneity of nutritional values is observed with, in particular, a significant difference in energy density among flours marketed in northern and southern countries. This analysis is part of a larger study which aims to understand the mechanisms of lipid oxidation in LC-PUFA-enriched infant foods.

**Polyunsaturated fatty acid biosynthesis in human CD3+ T cells is temporally related to cell activation.** Annette West, University of Southampton, United Kingdom; Johanna Von Gerichten, University of Surrey, United Kingdom; Nicola Irvine, University of Southampton, United; Kingdom; Elizabeth Miles, University of Southampton, United Kingdom; Karen Lillycrop, University of Southampton; United Kingdom, Philip Calder, University of Southampton, United Kingdom; Barbara Fielding, University of Surrey, United Kingdom; Graham Burdge, University of Southampton, United Kingdom
Polyunsaturated fatty acid (PUFA) biosynthesis has been reported in mixed leukocyte populations. However, the temporalrelationship between purified T cell activation and induction of PUFA biosynthesis is unknown.
To address this, the time course of n-6 PUFA synthesis, desaturase and elongase mRNA expression, and CD69 protein level weremeasured in CD3+ T cells from healthy volunteers (18–30 years; n=10).
Peripheral blood CD3+ T cells were incubated with or without concanavalin A (10μg/ml) in 10% (v/v) pooled plasma (18:2n-6:18:3n-3, adjusted to 10:1) with [d5]-18:2n-6 (4 μmol/L) for up to 48 hours. T cell fatty acid composition was determined by gas chromatography (GC) with flame ionisation detection. Isotopic enrichment was measured by GC-isotope ratio mass spectrometry. [d5]-Fatty acid concentration was calculated from the proportion of total fatty acid concentration.

mRNA expression was measured byquantitative RT-PCR, and CD69 expression by flow cytometry. Data were analysed by 2-way ANOVA.
CD69 expression was greater in stimulated than in quiescent cells at 14, 24 and 48 hours (8, 20 and 29–fold, time*activation $P < 0.0001$). Stimulation increased FADS2 mRNA expression at 14, 24 and 48 hours (5, 31 and 52-fold) (time*activation, $P < 0.0001$), aswell as FADS1 expression at 14 (1.5-fold), 24 and 48 hours (both 2.2-fold) (time $P = 0.001$). Stimulation did not alter ELOVL5expression. [d5]-20:2n-6 was the primary metabolite of [d5]-18:2n-6. [d5]-18:3n-6 was not detectable, while [d5]-20:3n-6 and[d5]-20:4n-6 were detectable but were < 0.001% of [d5]-18:2n-6. [d5]-20:2n-6 was undetectable at 14 hours,168 $\pm$ 9.9 fmol/106 cells at 24 hours and 413 $\pm$ 27 fmol/106 cells at 48 hours (time, $P=0.03$).
In conclusion, activated T cells increase PUFA synthesis via a mechanism that involves increased transcription of FADS2 andFADS1. 20:2n-6 is a major product of 18:2n-6 metabolism in stimulated human T cells and may act to negatively-regulateinflammatory response.

**Potential therapeutic applications of flaxseed peptides (linusorb).** Youn Young Shim, University of Saskatchewan, Canada; Ji Hye Kim, Sungkyunkwan University, Republic of Korea; Martin Reaney, University of Saskatchewan, Canada
Consumption of flaxseed (Linum usitatissimum L.) and numerous flaxseed products is associated with numerous health benefits. Flaxseed contains cyclic peptides (linusorbs, LOs), linked via an N-to-C terminal peptide bond, composed of eight to ten proteinogenic amino acids having molecular weights of approximately 1 kDa. [1–9-NαC]-linusorb B3 (a.k.a. cyclolinopeptide A) and [1–9-NαC]-linusorb B2 (a.k.a. cyclolinopeptide B), suppress immunity, induce apoptosis in human epithelial cancer cell lines, and inhibit T-Cell proliferation. The mechanism of linusorbs action is unknown. Using gene expression analysis in nematode cultures and human cancer cell lines we have observed that linusorbs exert their activity, in part, through induction of apoptosis. Specific linusorb properties include: 1) reversibly binding to human serum albumin; 2) induce heat shock protein (HSP) 70A production in Caenorhabditis elegans (exposure of nematode cultures to [1–9-NαC]-linusorb B3 (0.1 μM and 10.0 μM) induced a 30% increase in production of the HSP 70A protein); 3) induce apoptosis in human lung epithelial cancer lines; and 4) modulate regulatory genes in apoptosis in human lung epithelial cancer lines. These diverse activities indicate that linusorbs might induce apoptosis in cancer cells or act as versatile platforms to deliver a variety of biologically active molecules for cancer therapy. Surface plasmon resonance binding studies may aid in understanding the fate of linusorbs after consumption of flaxseed or flaxseed products or the development of linusorbs as drugs or drug carriers.

**Rapeseed and soy lecithin as vectors of α-linolenic acid: Impacts on adiposity, inflammation and gut microbiota in high-fat fed mice.** Marie-Caroline Michalski, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Chloé Robert, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Armelle Penhoat, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Nicolas Guillot, INSA-Lyon, France; Emmanuelle Meugnier, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Stéphanie Chanon, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Emmanuelle Loizon, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Aurélie Vieille-Marchiset, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; François Caillet, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Magali Monnoye, MICALIS Institute, INRAE, France; Hubert Vidal, CarMeN laboratory, Inserm U1060, INRAE UMR1397, France; Philippe Gérard, MICALIS Institute, INRAE, France; Carole Vaysse, ITERG, France
Justification: Dietary synthetic emulsifiers have recently been shown to promote metabolic syndrome and alter gut microbiota, while the effects of natural emulsifiers such as lecithin remain poorly described.
Objective: We evaluated, in mice, the impact of soy and rapeseed lecithin, both rich in essential α-linolenic acid (ALA), when incorporated in high-fat (HF) Western diets on ALA bioavailability, as well as on HF-induced adiposity, inflammation and gut microbiota.
Methods: For 13 weeks, male Swiss mice ($n=72$) were fed a Chow diet, a control semi-synthetic HF-diet (25wt % lipids) poor in ALA (HFC), or different ALA-enriched (4.7wt% of total fatty acids) HF-diets containing 0% lecithin (HFA-L0), a nutritional dose of soy or rapeseed lecithin (10wt% of lipids; HFA-SL10%, HFA-RL10%), or a 20wt% supplemental dose of RL (HFA-RL20%). We analyzed histomorphology of the epididymal adipose tissue (EAT), hepatic lipid composition by GC-FID, gene expression by RT-PCR, and faecal microbiota composition by 16S sequencing.
Results: Within ALA-rich HF diets, the hepatic bioavailability of ALA was similar whether ALA was vectorised as lecithin (HFA-RL10%, HFA-RL20%, HFA-SL10%) or as oil only (HFA-L0) (yet, all higher than HFC). Similarly to HFC, HFA-SL10% and HFA-RL20%, but not HFA-RL10%, increased body weight gain ($p< 0.001$), visceral adiposity ($p< 0.001$) and adipocyte hypertrophy ($p< 0.05$), vs Chow. Lecithin in HF-diets, regardless of origin or dose, cancelled the anti-inflammatory effect of ALA, observed in HFA-L0, on the expression of genes involved in macrophage infiltration in the EAT (e.g., Tnfα, Cd11c). Only HFA-RL10% increased gut microbiota α-diversity vs HFC ($p< 0.05$), and altered the abundance of Lachnospiraceae and Desulfovibrionaceae.

Significance to the AOCS membership: At a nutritional dose, rapeseed lecithin, unlike soy lecithin, did not significantly enhance visceral adiposity comparatively to a chow diet, and increased gut bacterial diversity. Rapeseed lecithin may therefore be considered as a promising natural lipid emulsifier.

**Supplementing docosahexaenoic acid along with arachidonic acid during weaning period improves immune response in neonatal Brown Norway rats.** Dhruvesh Patel, University of Alberta, Canada; Marnie Newell, University of Alberta, Canada; Susan Goruk, University of Alberta, Canada; Sue Tsai, University of Alberta, Canada; Caroline Richard, University of Alberta, Canada; Catherine Field, University of Alberta, Canada

As infant's immune system matures, T helper type-2 (Th2; interleukin (IL-4 & IL-13) response becomes Th1 dominant with higherinterferon-γ (IFNγ) & tumor-necrosis-factor-α (TNFα). Although supplementation of fatty acids (FA) arachidonic (ARA) anddocosahexaenoic (DHA) influence the immune development, its role in infants with genetic susceptibility to allergies (Th2 skewedimmune condition) is unknown. We aim to study the effect of ARA+DHA supplementation during suckling and weaning period onimmune system development of Th2 skewed Brown Norway rats.

Pregnant rats received nutritionally complete high-fat diets (MD); ARA+DHA (0.4%ARA+0.8%DHA, w/w total fat) or control diet (0% ARA+DHA) from 5d preparturition to the end of suckling (3wk). Using crossover design, half pups from each MD groupsreceived either ARA+DHA (0.5%DHA & ARA each, w/w total fat) weaning diet (WD) or control WD for 4–8wk. At 8wk, pups werekilled to collect plasma and spleen. FA composition in phospholipids (PL), phenotype and ex-vivo cytokine production to mitogens (phorbol-myristate-acetate+ionomycin; PMAi, lipopolysaccharide; LPS) were measured in isolated splenocytes.

Pups' food-intake, bodyweight and splenocyte number did not differ due to diet. Compared to controls, changes in PL FA due to MDARA+DHA were not present at the end of weaning, yet MD had a programming effect on splenocytes composition; higher adaptivecells, OX12+ B cells and FoxP3+CD25+CD4+CD3+ regulatory T cells, and lower innate cells (CD11+ monocytes) $p < 0.05$. WDsupplementation resulted in significantly higher DHA (1.5x) in PL of spleen and plasma, without changing ARA content. PMAistimulated splenocytes from ARA+DHA WD pups resulted in 50% more IFNg and TNFα (Th1) compared to control WD ($p < 0.05$).

Using allergic rodents, we showed the importance of dietary ARA+DHA and its effects on immune development. MDsupplementation had programming effect on adaptive immune cells and WD supplementation enhanced Th1 type immune response toPMAi.

**Tribo-rheological analysis of reconstituted gastrointestinal porcine mucus under different pH conditions.** Gustavo Ruiz-Pulido, Tecnologico De Monterrey, Mexico; Dora Medina, Tecnologico de Monterrey, Mexico

The project analyses the physicochemical and mechanical properties of pig gastrointestinal mucus from a rheological point of view. Considering that mucus is a viscoelastic protective barrier that covers the internal walls of gastrointestinal tissues, developing fundamental functions as: protecting of the body from the entrance of environmental pathogens and toxins, lubricating of the epithelium to reduce mechanical stress (shear strength) caused by swallowing food, avoiding excessive water loss to maintain hydration, and shielding the stomach from gastric acids. The viscoelastic and protective properties of mucus are mainly produced by a mucin network, stabilized through electrostatic, hydrophobic and hydrogen bonding interactions. Mucin is a glycoprotein with a polyanionic nature at physiological pH, which is determinant in mucus rheology. At neutral pH, mucus presents viscous behavior produced by chains crosslinking. Instead, at acidic pH, mucus acquires an extended conformation related to an elastic behavior produced by mucus gelation in the stomach. Therefore, rheological analysis is important to visualize changes in viscosity, friction coefficient, resistance to different shear stresses and oscillatory stress to which the intestinal mucus is subjected under natural conditions, considering the different pH values of the gastrointestinal.

**Uptake of fatty acids by the enterocyte: New insights gained from mathematical modeling.** Julie Etienne, Inserm U1060, INRAE UMR1397, UCB Lyon 1, INSA-Lyon, Inria, France; Armelle Penhoat, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Marie-Caroline Michalski, Inserm U1060, INRAE UMR1397, UCB Lyon 1, France; Carole Knibbe, Inserm U1060, INRAE UMR1397, UCB Lyon 1, INSA-Lyon, Inria, France

Justification: After a meal, fatty acids (FA) released from digested dietary triglycerides, are taken up by absorptive cells, namely intestinal enterocytes. This step is usually reviewed as being composed of passive diffusion along with a carrier-mediated pathway. However, in the absence of a clearly identified membrane carrier, it remains unclear whether the latter actually exists.

Objective: We aim at testing, using mathematical modeling and data fitting, whether the observed dose-response curves of apical enterocyte FA uptake are actually compatible with the pathways suggested by the literature. We propose relevant alternative mechanisms.

Methods: Uptake of FA by the enterocyte was first modeled as passive diffusion only (model M0) and studied theoretically. Two other models were implemented: the one supported by literature with both passive

diffusion and a carrier protein (M1); an alternative one with passive diffusion followed by enzymatic transformation inside the cell (M2). We compared these models to experimental data from the literature on rodents and Caco-2 cells.
Results: The analytical study of M0 allowed us to (1) demonstrate that the dose-response curve depends much more on volume ratios than on permeability values, (2) predict the dose-response curve for a wide range of realistic volumes, and (3) show that even the highest values cannot produce a high enough uptake response. The active mechanisms added in M1 and M2 allowed the response to reach experimental data. Both M1 and M2 fitted the data equally well. However M2 has a much larger uptake potential for the same protein parameters, thereby suggesting that it is more efficient for the cell to invest in an intracellular enzymatic transformation than in a membrane carrier.
Significance to AOCS: Interdisciplinary, quantitative mathematical modeling may help targeting molecular candidates and guide experimental effort to gain insight on physiological mechanisms of importance in the health impact of dietary lipids.

***Abstracts for the following Health and Nutrition presentations were not provided:***

**Students' Perspectives: The Pros and Cons of Online Learning.** Jessica Ulbikas; Celeste Chadwick
Modeling the Impact of High Oleic Acid Oil Varieties on The Levels of Essential Fatty Acid Intake in Children. Martha Belury, The Ohio State University, USA

## Industrial Oil Products

*Division Vice Chair: Brajendra Sharma, University of Illinois, USA*

### General Industrial Oil Products

*Chairs Darrell Sparks, Mississippi State University, USA; Brajendra Sharma, Prairie Research Institute-Illinois Sustainable Technology Center, University of Illinois at Urbana-Champaign*

**Determination of Estolide Molecular Weight Distribution via Gel Permeation Chromatography.** Grigor Bantchev, USDA-ARS-NCAUR, USA; Steven Cermak, USDA-ARS-NCAUR, USA; Amber Durham, USDA-ARS-NCAUR, USA; Neil Price, USDA-ARS-NCAUR, USA
Using the universal calibration and the Mark- Houwink equation (MHE) ($[\eta]j=K(Mj)\alpha$ ), three batches of oleic estolide acids and their corresponding 2-ethylhexyl esters were characterized using gel permeation chromatography (GPC). The MHE parameters in tetrahydrofuran (THF) at 40 °C were determined (for acids: $\alpha = 0.442\pm0.003$ and $\log K=2.505\pm0.007$, for esters: $\alpha =0.531\pm0.006$ and $\log K =2.79\pm0.02$). The fits of the GPC chromatograms yielded also the oligomeric composition of the estolides, which can be used to calculate the estolide number (EN) of an estolide mixture, and other molecular-weight distribution parameters, such as number-average molecular weight (Mn), weight-average molecular weight (Mw), and dispersity (Đ). Using the Deming line fit, we concluded that the GPC should be expected to be approximately three times more sensitive than the currently used methods for determination of EN values.

**Developing High-oleic Oil of Yeasts as Potential Feedstock to Produce Biolubricant.** Mahesh Khot, University of Concepcion, Chile; David Contreras, University of Concepcion, Chile
The exposure of conventional mineral lubricants to the environment and their entry into aquatic bodies lead to damage to the ecosystem and health hazards to living beings. As a result, bio-based lubricant formulations such as environmentally acceptable lubricants (EALs, biolubricants) are gaining considerable attention, associated with biodegradable and nontoxic nature. Pre-existing alternatives include vegetable oils and constitute the most common category of lube base oils of EALs. However, several drawbacks, namely high cost, poor low-temperature fluidity, and thermo-oxidative instability, compromise lubricant application performance.
With sweeping global environmental regulations, there is a growing concern for mineral oil-based lubricants' sustainability for automotive/industrial applications. There is a dire need to shift toward sustainability and use green chemistry to derive new base oils from renewable resources. As the focus is on reducing petroleum use, its availability will continue to decrease for the lubricants; so there needs to be a replacement for this petroleum volume loss. These shortcomings can be addressed by applying triacylglycerol accumulating fungi to evaluate their lipids as a base oil for biolubricant formulation.
A bioprocess is proposed to cultivate yeast biomass on the renewable substrate (lignocellulosic pentosan derived from sugarcane bagasse) followed by extraction of total cellular lipids as base oil. Fungal lipids with a synergistic mixture of saturated/unsaturated fatty acids would represent a better alternative to mineral and vegetable oil-based lubes. Many oleaginous strains produce high oleate oils and can be engineered to deliver the desired fatty acid profile. High-oleate content would render the yeast oil suitable for lubricant applications that require high oxidative stability. This new lubricant formulation is expected to meet ecotoxicological properties. Being fatty-acid based, there will be ultimate biodegradation (total mineralization) or short persistence in the environment when spilled.

**Solubility of Unsaturated Fatty Acids in Deep Eutectic Solvents.** Adeeb Hayyan, University of Malaya, Malaysia; Mohamed Hadj-Kali, King Saud University, Saudi Arabia; Maan Hayyan, Muscat University, Oman; Ainul F. Kamarudin, University of Malaya, Malaysia; Hanee Hizaddin, University of Malaya, Malaysia; Emad Ali, King Saud University, Saudi Arabia; Mohd Ali Hashim, University of Malaya, Malaysia

Deep eutectic solvents (DESs) are considered solvents of the 21st century. They are alternatives to organic conventional solvents. DESs have been deemed to be "super fluids" due to their wide range of applications such as biodiesel production, treatment of low-grade palm oil, extraction of pectin from peels of fruits and enhancement of lipase enzymes. DESs have negligible vapor pressure, high thermal and chemical stability, wide electrochemical window and they are tailorable to desired applications. In this study, solubility of unsaturated fatty acids (linoleic acid and oleic acid) in deep eutectic solvents was investigated. Computational and experimental work were conducted using COSMO-RS which represents Conductor-like Screening Model for Real Solvents. COSMO-RS thermodynamic model based on the quantum chemical calculations. It normally acts as a bridge between both quantum chemistry and chemical engineering thermodynamic. COSMO-RS is fast and reliable computational approach for the solvent screening because it is mainly based on the prediction of the thermodynamic properties and behavior of un-saturated fatty acid solubility in DES. The interpretation of the σ-profiles, as well as the evaluation of Gibbs energy change of mixing were in excellent agreement with the experimental results. This study can could introduce a new perspective in oil extraction and improvement of industrial oils products.

**That's a RAP: Improving the Recyclability of Asphalt Pavements with Soybean Oil-based Polymers and Rejuvenators.** Eric Cochran, Iowa State University, USA; R. Chris Williams, Iowa State University, USA; Baker Kuehl, Iowa State University, USA; Austin Hohmann, Iowa State University, USA; Maxwell Staver, Iowa State University, USA

Pavements are energy intensive materials, requiring about 4 trillion Joules (TJ) per ton, equating to the combustion of about 100 tons of crude oil. While asphalt pavements are only slightly less energy intensive compared to Portland cement concrete, they offer a far greater potential for recycling. RAP (recycled/reclaimed asphalt pavement) is generated during a "mill-and-fill" construction operation where an end-of-life asphalt pavement is ground into piles of aggregate bound by aged and oxidized asphalt binder. The degree to which RAP can be incorporated into new pavements is largely limited by its brittleness, driven by the aged binder at a molecular level through its oxidized asphaltene aggregates. To realize the cost and energy savings promised by asphalt recycling, additives known as "rejuvenators" are needed that restore virgin-like material properties to high-RAP mix designs. In this contribution we introduce sub-epoxidized soybean oil (SESO) as a Reactive Restorative Modifier (RRMs), a new class of asphalt rejuvenator that functions through its reactivity with aged asphaltenes. By exploiting the degree of epoxidation as a design variable, RRMs with optimized viscosity, solvency, and reactivity disperse and conjugate with asphaltenes during hot mix production, preventing their reagglomeration to yield pavements that meet or exceed a wide variety of industry standards including, Ideal-CT, I-Fit, DCT, and Hamburg Wheel Tracking. SESO can also be used as a solvent to manufacture poly(acrylated epoxidized high oleic soybean oil) (PAEHOSO), which further adds the elastic properties required for heavily-trafficked pavements. We expound upon these claims through several laboratory and field experiments. For example, in Indiana we used SESO to produce a 40% RAP mix with a DCT value of 507 J/m2 and an I-Fit value of 12.6, far exceeding common minima of 400 J/m2 and 8.0, respectively. Our results illustrate excellent potential for economic development and positive environmental impacts.

**The Effect of Fatty Acid Unsaturation on Properties and Performance of Monomers, Polymers, and Latexes from Plant Oils.** Zoriana Demchuk, Oak Ridge National Lab, USA; Oleh Shevchuk, Lviv Polytechnic National University, USA; Vasylyna Kirianchuk, Lviv Polytechnic National University, USA; Ananiy Kohut, Lviv Polytechnic National University, USA; Stanislav Voronov, Lviv Polytechnic National University, USA; Andriy Voronov, North Dakota State University, USA

Renewable raw resources, commonly vegetable oils, have become progressively interesting in making bio-based polymers and polymeric materials because of low cost, positive environmental impact, and rich application possibilities. Recently, a library of acrylic monomers from a variety of plant oils (POBMs) was synthesized using a one-step transesterification reaction of vegetable oil triglyceride with N-hydroxyethyl acrylamide. POBMs perform themselves as conventional vinyl monomers in free radical homopolymerization and copolymerization reactions. The impact of the unsaturation amount of plant oils chosen for monomer synthesis has a noticeable effect on the macromolecule length and polymerization rate of the resulted plant oil-based monomers. The fascinating structure of plant oil-based monomers provides the opportunity for crosslinking reactions using double bonds of fatty acid chains. Moreover, using 1H NMR spectroscopy it was established that double bonds of fatty acid fragments remain mostly unaffected during the polymerization and therefore provide the formation of crosslinked polymer networks via autooxidation mechanism.

Stable latexes with high POBM content were synthesized in copolymerization with a range of comonomers (renewable plant-based and commercially available petroleum-

based) using miniemulsion polymerization. The mechanical properties of latex materials were evaluated using DSC, DMA, tensile, and pendulum hardness testing.
The glass transition temperature (Tg) noticeable decreases with the increasing amount of POBM in reaction feed composition. The established linear dependence of the crosslink density on monomer feed unsaturation allows adjusting the thermomechanical properties of the latex networks by merely mixing various plant oil-based monomers at certain ratios in the monomer feed. Besides, the presence of plant oil-based fragments in the macromolecular backbone provides the plasticizing and hydrophobizing effect, as it was shown by increasing the water contact angle of latex films made from soybean oil-based monomer copolymerized with Vac and MMA. The latter results make the biobased latexes potential candidates for coatings and adhesives applications.

**Unique Properties and Applications of Guerbet Alcohols and Their Ethoxylate Derivatives.** Ramesh Varadaraj, Sasol North America, USA; Ollie Normand, Sasol, USA; Dustin Landry, Sasol, USA
This presentation will discuss the unique properties and novel applications of Guerbet alcohols and their ethoxylate derivatives. The broad matrix comprising Guerbet alcohols with carbon numbers from 12 to 32, and Guerbet alcohol ethoxylates with 3 to 150 mole ethylene oxide (EO) will be discussed. Compared to their linear counterparts, Guerbet alcohols exhibit significantly lower melting points, viscosity and pour points, and higher oxidative stability, surface wettability and lubricity. The Guerbet ethoxylates exhibit marked difference in physical and interfacial properties such as critical micelle concentration, effectiveness of surface tension reduction, foaming and wettability compared to their respective linear counterparts. The presentation will focus on illustrating how the β- branched Guerbet molecular structure influence physical and interfacial properties. And, how the unique properties translate to enhanced performance in applications such as, metal working lubricants, engine lubricants, cosmetic formulations, hard surface cleaners, defoamers, and crude oil pour point depressants. Thus, a molecular structure- physical property- applications performance relationship will be presented to highlight for what applications the Guerbet hydrophobe can enjoy a preferred status.

## Green Chemistry and Oleochemicals

*Chairs Majher Sarker, ARS/USDA, USA; Helen Ngo, USDA ARS ERRC, USA*

**Continuous Reactive Extraction for Manufacturing of CMF and HMF from Fructose with Milli-/microstructured Coiled Flow Inverter.** Frank Schael, Darmstadt University of Applied Science, Germany; Krishna Nigam, Indian Institute of Technology, India; Patrick Rojahn, Hochschule Darmstadt University of Applied Science, Germany
The chemical catalytic approach to manufacture value-added chemicals from biomass and biobased feedstocks is currently widely investigated due to possible advantages over microbial and thermo-chemical processing. In this framework 5-(hydroxymethyl)furfural HMF and 5-(chloromethyl) furfural CMF attracted a lot of attention in recent literature as potential candidates for new platform chemicals.
HMF and CMF are obtainable by conversion of sugar and sugar containing waste streams. The underlying technical process can beneficially be performed by combining the two-unit operations reaction and extraction in line with a green engineering principle. Recent progress of milli-/microstructured reactors and other miniaturized equipment opens the pathway to energy and resource efficient process development and decentralized chemical manufacturing due to superior mass, energy, space efficiency.
In this work the continuous flow synthesis of HMF and CMF is investigated by means of a reactive extraction using a coiled flow inverter reactor concept which consists of helically coiled tubular reactors bended by 90° at regular spatial distances. The low-cost reactor concept is well-known for its superior narrow residence time distribution, heat and mass transfer capabilities when compared to helically coiled reactors for relatively low Reynolds numbers.
The continuous flow setup allowed determination of HMF and CMF yields for various residence times, temperatures, fructose concentrations, reactor sizes and phase ratios. Three solvents (cyclopentyl methyl ester, anisole, and toluene) were investigated which are considered as less problematic as the halogenated solvents previously employed for this process. The slug flow regime concept provided efficient continuous flow extraction of CMF. CMF yields of up to 65% for residence time of 2.5 minutes were observed and solvent polarity was found to be among the most important solvent parameters.
The results obtained show that the continuous flow setup allows time effective process development studies for a more environment-friendly process.

**Design of Sustainable Chemistry to Produce Functional Bioactive Fatty Acid Products.** Helen Ngo, USDA ARS ERRC, USA; Xuetong Fan, USDA ARS ERRC, USA; Richard Ashby, USDA ARS ERRC, USA
Petroleum-based materials, while generally cheaper to produce than biobased products, tend to resist the typical degradation processes found in nature, resulting in global problems with waste management. Biobased products, on the other hand, have an established track record as reliable and low-carbon footprint replacements for many petroleum-based products. This

presentation will focus on the design of sustainable chemistry to create a family of unique biobased fatty acid products exhibiting effective functional (phenolic) groups against bacteria. The enhanced bioactive properties come from the hydroxyl functional group on the aromatic ring as it improves the hydrophilicity of the fatty acid. The paper will also discuss the hurdles encountered in the process design of these products. This development will result in a renewable family of products replacing petroleum-based materials in the antimicrobial monomer and polymer areas.

**Development of Soluble Soybean Polysaccharide/ agar Blend Film for Edible Oil Packaging.** Jie Liu, Zhengzhou University, China (People's Republic); Chang Liu, Zhengzhou University, China (People's Republic); Xuejing Zheng, Zhengzhou University, China (People's Republic); Keyong Tang, Zhengzhou University, China (People's Republic)

Bio-based polymers have attracted increasing attention in edible oil packaging, because they could alleviate the serious environmental problems caused by the fossil-based plastic packaging. In the present work, a novel blend film was developed from soluble soybean polysaccharide (SSPS) and agar, which is a potential candidate for edible oil packaging. The structure, mechanical, barrier, and physical properties of the SSPS/agar film were investigated and the oxidative stability of olive oil in the blend film was also evaluated during storage. SSPS/agar film exhibited significantly higher tensile strength and UV barrier property but lower elongation at break, compared to pure SSPS film ($p < 0.05$). Results also showed that all of the blend films had good anti-oil properties. Based on ATR-FTIR spectra, strong intermolecular interactions between SSPS and agar macromolecules were found in the blend films. It was also observed from SEM micrographs that the composite films show heterogeneous structure. The peroxide value of the olive oil stored in heat-sealed SSPS/agar pouches increased rapidly during the initial 5 days of storage (conditions: 30 °C, 57±2% RH), but increased relatively slow up to 30 days of storage. The results of the present study suggest that the obtained SSPS/agar blend films may have great potential in edible oil packaging for extending the shelf life of oil-containing food products.

**Green Process for Selective Ester Production Using Heterogeneous Resin Catalyst with Different Reaction Field.** Kousuke Hiromori, Tohoku University, Japan; Kazuki Murakami, Tohoku University, Japan; Atsushi Takahashi, Tohoku University, Japan; Naomi Shibasaki-Kitakawa, Tohoku University, Japan

Propylene glycol fatty acid esters consist of fatty acid and propylene glycol. Monoester and diester have different functions depending on number of fatty acids bonded. These are industrially produced by esterification with homogeneous acid catalyst under severe conditions (170–210°C, vacuum). Because the esterification proceeds sequentially, the product becomes a mixture of mono- and di-esters. Thus, it is necessary to separate not only the catalyst but also each ester, so the complicated process with large environmental impact is necessary.

As an alternative, we have proposed a process for selectively producing each ester using heterogeneous resin catalyst swollen by propylene glycol or fatty acid. Based on the previous work in the batch system, a continuous production process with two columns packed with the resin swollen by each reactant connected in series was designed. In this process, monoester-rich product was obtained from the first column and diester-rich product was obtained from the second column under mild condition (below 80°C, atmospheric pressure) and the desired esters selectivity at each column were significantly improved compared with those in conventional process (30–50%). Additionally, the repeated use of the catalyst, which is a feature of heterogeneous catalysts, was also investigated. As a result, it was found that the resin catalyst would be used repeatedly without loss of activity.

**Impact of Biochemical Stimulants on Lipid and DHA Production by aurantiochytrium Sp.** Majher Sarker, ARS/USDA, USA; Syed Hussain, ARS/USDA, USA; Hailemichael Yosief, ARS/USDA, USA; Alexander Bassett, Rowan University, USA

The impact of five different chemicals; 2-phenylacetic acid (PAA), humic acid (HA), spermidine (SPD), 1-methylcyclopropene (1-MCP), and ferric chlorides (FC), on production of lipid and docosahexaenoic acid (DHA) using Aurantiochytrium sp. ATCC PRA-276 was evaluated. After 120 h of cultivation, the individual supplementation of PAA, HA, and FC, increase the biomass, total lipid and DHA production of Aurantiochytrium PRA-276 by 32%–76%, 23%-34%, and 53%–86% in comparison to the control, respectively. The highest biomass and DHA productivities were observed with the supplementation of PAA and FC achieving 76% and 67% and 86% and 66%, compared to the control, respectively. This study suggests that the introduction of aforementioned bio-stimulants can increase the productivity of Aurantiochytrium sp to obtain better yields of lipid and DHA which will lead to economic-feasibility for large scale production.

**Nature-inspired Resins for Additive Manufacturing.** Joseph Stanzione, Rowan University, USA

Vinyl ester resins (VERs) are used to produce polymers for a variety of commercial and military applications due to their relatively high strengths, moduli, thermal stabilities, and chemical resistances. Most commercially available VERs are petroleum-based and are typically cured into polymers via traditional manufacturing

techniques, such as resin transfer molding, compression molding, and thermal curing. In an effort to move towards sustainability, bio-based feedstocks have regained considerable interest for the development of such polymers. To achieve similar properties to current, industrial VERs, bio-based resins need to possess intrinsic molecular characteristics (i.e., a balance between aromatic and aliphatic contents assembled in strategic methods) to impart favorable processing and performance attributes. Furthermore, additive manufacturing (AM), also known as 3D printing, has gained significant traction as a favorable manufacturing technique over traditional methods due to the ability to create customizable parts with reduce wastes.

In this study, relatively low viscosity (< 1,000 cP at 25°C), bio-based VERs were synthesized in solventless, two-step, 100% atom efficient reactions. Photorheology, along with measured photocured parameters, were utilized to determine the feasibility of the resins to be polymerized using stereolithography (SLA), a 3D printing method. The bio-based VERs were cured via SLA with extents of cure of > 95%, after a mild post cure. Viscoelastic, thermogravimetric, and mechanical testing were performed to determine polymer performance. Dependent on resin formulation, the SLA printed VERs exhibited glass transition temperatures ranging from 11°C to 120°C, thermal stabilities ≥ 300°C, tensile strengths ranging from 12 MPa to 20 MPa, and critical strain energy release rates ranging from 160 J m-2 to 730 J m-2. This study has shown that by strategically combining molecular building blocks derived from various bio-based feedstocks, namely lignin, cashew nut shell liquid, and carbohydrates, thermosetting SLA polymers with desirable processing and final product properties can be achieved.

## New Uses of Glycerine

*Chair Franck Dumeignil, University of Lille / UCCS, UMR CNRS 8181, France; Dharma Kodali, University of Minnesota, USA*

**Biobased Glycerol: The Profitable Platform Biochemical of the Chemical Industry.** Mario Pagliaro, CNR, Italy

Biobased glycerol, namely glycerol by-product of vegetable oil transesterification with methanol (to make biodiesel) and hydrolysis (to make soap), is the main platform biochemical of the chemical industry. Its low cost and increasing abundance due to the booming biodiesel production started in the late 1990s led to the introduction of several new catalytic processes that today enable the profitable production of numerous commodity and specialty chemicals starting from glycerol rather than from propylene. Furthermore, glycerol today successful replaces functional petrochemicals used as cement multipurpose aid in the construction industry, or as antifreeze in cold areas of the world. Biocompatible glycerol-based polymers, too, emerged as important alternatives to conventional polymers in the biomedical and patient care markets.

Which are the main chemicals and polymers produced today from glycerol? Which conventional petrochemicals are being displaced from bioglycerol-derived products? Do all productions necessarily require high purity glycerol? Who are the main players in the glycerol biorefinery industry?

This lecture will answer these and related questions offering an industrially relevant perspective given by an eminently practical viewpoint.

**Continuous Flow and Glycerol—Recent Impacts of Innovative Technologies.** Christophe Len, Chimie Paristech, France

The design of environmentally friendly methodologies has been the driving force of scientists in recent years. In particular, the use of biomass-derived materials, green solvents and continuous flow as an alternative technique has been investigated. In this regard, glycerol has the potential to be both an excellent renewable solvent in modern chemical processes and a versatile building block in biorefineries. Various chemical transformations such as oxidation, hydrogenolysis, etherification, esterification, dehydration and oligomerization can be executed to create a large number of value-added chemicals with specific applications in the polymer, agrochemical, and pharmaceutical industries. In recent years, continuous flow and continuous processing offer significant processing advantages compared to the traditional batch chemistry; the main improvements being thermal management, mixing control, adaptation to a wider range of reactions conditions, scalability, energy efficiency, waste reduction, safety, use of heterogeneous catalysts, multi-step synthesis, among others. Recent advances in glycerol valorization to valuable products (acrolein, lactic acid, glyceric acid, propanol, propanediols, glycerol carbonate, solketal, acetin, and oligomers) under liquid phase continuous flow systems using different types of catalysts and processes will be highlighted [2].

[1] C. Len and coll., J. Agric. Food Chem. 1996, 44, 2856; 1997, 45, 3; Green Chem. 2011, 13, 1129; Eur. J. Org. Chem. 2013, 2583; Catal. Commun. 2014, 44, 15; RSC Adv. 2014, 4, 21456; Catalysis Today 2015, 255, 66; J. Chem. Technol. Biotechnol. 2017, 92, 14; ACS Sustainable Chem. Eng. 2016, 4, 6996; Catalysts 2017, 7, 123; Molecules 2019, 24, 1030; Front. Chem. 2019, 7, 357.

[2] C. Len and coll., J. Ind. Eng. Chem. 2017, 51, 312; Synthesis, 2018, 50, 723; Curr. Opin. Green Sustain. Chem. 2019, 15, 83.

**Conversion of Glycerol to Glycidyl Ether: Selective Catalytic Process Toward Value-added Chemicals.** Yoshihiro Kon, National Institute of Advanced Industrial Science and Technology (AIST), Japan; Benjamin Katryniok, Centrale Lille, France

Producing useful chemicals from glycerol is of great industrial importance. We have developed a three-step catalytic conversions of glycerol to glycidyl ether via allyl alcohol and allyl ether as intermediates. Each step was carried out by heterogeneous catalysts such as Re-Al2O3, MoO3-TiO2, and TS-1 zeolite to give the desired products in around 90% yields for each step. These reactions could significantly increase the economical viability of the biodiesel value chain by producing a value-added materials for electronic devices. Moreover, these new methods would reduce the environmental impact of allyl alcohol production.

**Identification of the Active Phase in Ca-based Glycerol Polymerization Catalysts.** Franck Dumeignil, University of Lille / UCCS, UMR CNRS 8181, France; Negissa EBADI POUR, University of Lille, France; Benjamin Katryniok, Centrale Lille, France; Laurent Delevoye, CNRS, France; Betrand Revel, University of Lille, France; Sébastien Paul, Centrale Lille, France

Glycerol polymerization was performed over several heterogenous catalysts including alkaline earth oxides-based ones. Ca-based catalysts were of specific interest due to their higher performances. We analyzed the spent catalysts obtained from calcium oxide and calcium hydroxide solids using X-ray powder diffraction (XRD) and solid-state NMR. From the XRD results, it was evidenced that calcium glycerolate was present as a crystalline phase in spent catalysts. Moreover, the results of 13C solid-state NMR suggested two possible structures for spent catalysts: a linear structure with the Ca(C3H7O3) formula and a cyclic-branched one with the Ca(C3H6O3) formula. When using the CaO spent catalyst in recycling tests, a glycerol conversion of 47% (vs. 22% for the fresh catalyst) was observed at 245 °C after 4 h using 20 mol of glycerol as a starting material in the presence of 0.7 mol of catalyst. Further, a higher degree of polymerization was observed with the spent catalyst (70% selectivity to tetramers and higher polymers vs. 55%). XRD and solid-state 13C-NMR analyses together with catalytic tests' results suggest that Ca-glycerolate is actually the solid active phase that is formed in situfrom calcium oxide or hydroxide. Furthermore, we propose a mechanism involving the dissolution of CaO, the formation of Ca-glycerolate and its precipitation followed by crystallization.

**Kinetic Model for Process Design of Efficient Production of High Value-chemicals from Glycerol.** Tsutomu Chida, Tohoku University, Japan; Kousuke Hiromori, Tohoku University, Japan; Naomi Shibasaki-Kitakawa, Tohoku University, Japan; Naoki Mimura, National Institute of Advanced Industrial Science and Technology (AIST), Japan; Aritomo Yamaguchi, National Institute of Advanced Industrial Science and Technology (AIST), Japan; Atsushi Takahashi, Tohoku University, Japan

One of the effective ways to use glycerol (G) is the conversion into glyceric acid (GA), which can be used as a pharmaceutical raw material. This reaction proceeds using an Au catalyst in the presence of NaOH. However, many side reactions proceed simultaneously, so that GA yield is around 50%. In order to achieve an efficient GA production, it is important to elucidate the detailed reaction mechanism and to construct a kinetic model to predict GA yield under various conditions. Our previous study clarified that GA was formed via two steps: activation of glycerol into glycerol alkoxide by a strong base in liquid phase and reaction of glycerol alkoxide with reactive oxygen species OOH on the Au catalyst. In this study, a kinetic model based on the mechanism was constructed to describe the reaction behavior under various conditions of pH, partial oxygen pressure (PO2), catalyst amount and Au ratio.

**Promising Use of Glycerol as a Reagent and a Solvent.** Karine De Oliveira Vigier, Cnrs-Universite De Poitiers- IC2MP, France

Glycerol is a widely used chemical that can be converted to a wide range of molecules. One interesting reaction from glycerol is oligomerization reaction leading to oligo-glycerols. These compounds are an interesting class of chemicals that exhibit a broad range of applications in various industrial sectors such as cosmetic, food and pharmaceutic industries. This is a challenging reaction mainly because of the difficulty to closely control the reaction selectivity. Oligomerization of glycerol was performed in the presence of acid (heterogeneous or homogeneous) catalysts. The esterification of glycerol with betaine hydrochloride, a bio-based compound coming from the sugar beet industry to produce ionic surfactants or liquids was also investigated through DFT modelling and experimental approaches. The absence of an external catalyst and solvent, as well as the use of a stoichiometric mixture of acidified glycine betaine and glycerol, two co-products of the sugar beet and vegetable oil industries, respectively, represent notable advantages within the framework of sustainable chemistry. Another interesting reaction is the etherification of glycerol with short chain alkyl in the presence of homogeneous catalyst. Among the large variety of Brønsted and Lewis acids tested, metal triflates were not only the most active ones but were also capable of catalyzing this reaction with a high selectivity. Glycerol was also studied as a solvent in the conversion of carbohydrate to furanic derivatives. We have shown that glycerol or glycerol carbonate could replace ionic liquids (IL) in the conversion of fructose to 5-hydroxymethulfurfural,

an interesting platform molecule. All the results obtained during these studies will be presented.

## Biofuels

*Chairs Robert Dunn, USDA ARS NCAUR, USA; Bruce Patsey, Oil-Dri, Corp., USA*

**Biodiesel's Role in the Future Alternative Fuel Scenario.** Martin Mittelbach, Universtity of Graz, Austria
Globally Biodiesel today is the most important alternative Diesel fuel. Especially countries with high production of corresponding feedstocks today are leading in biodiesel utilization according to mandates. So, Indonesia introduced a mandatory B30 in 2020 and Brazil will increase the mandatory biodiesel share up to 15 % in 2023. In the United States, biodiesel and HVO production was a record of around 7.5 Mill tons in 2019. The still leading region for biodiesel and HVO is the European Union with around 14 mill tons in consumption. However, in this region the utilzation of biodiesel is stagnating, mainly because of limited agricultural areas, so a considerable part of feedstocks and fuel has to be imported. As in Europe the mandatory blend of biodiesel in passenger cars is limited to B7, the share of drop-in fuels like HVO is rising leading to competition with oil and fat feedstocks. As consequence of limited agricultural areas and negative image of ''bio'' fuels decarbonization is favored promoting electromobility as well as non biomass based fuels like E-fuels or power to X fuels. However, those strategies include high investment and production costs, so it can be questioned if in the near future considerable amounts of those fuels will be available.

**Biofuel Transformation of the Heating Market.** Thomas Butcher, National Oilheat Research Alliance, USA
The oilheat industry in the U.S. serves about 5 million homes- mostly in the Northeast but also in the Mid-Atlantic and Pacific Northwest. As in other sectors of the national energy market, there is strong motivation to move building heating to being nearly 100% renewable. While much of the attention is on electric heat pumps and renewable electricity, the use of biofuels in this market could achieve dramatic reductions in greenhouse gas emissions with lower investment cost. NORA is supporting the industry in developing a path to 100% biofuels by 2050.
Several different fuels are under consideration including hydrogenated vegetable oil (HVO), gas-to-liquid (GTL) fuels, and ethyl levulinate. However, biodiesel is currently the most available and lowest cost option. Advantages and disadvantages of these options will be discussed. Technical areas of focus in the broad conversion from petroleum fuels to biodiesel include cold flow concerns, long term storage stability, and compatibility with common elastomers.
The range of cold flow properties of commercial biodiesel fuels, the potential for improvements, and the in-field hardware implications of the cold flow concerns will be presented. To compliment lab stability studies, NORA is undertaking long-term in-field studies of changes in fuel properties over time in the specific environment of the heating sector. Also to be presented is discussion of the range of elastomer types in legacy and new (bio-specific) system hardware and the compatibility with biodiesel. NORA has recently completed an extended fuel pump trial which provides encouragement about durability with this new fuel.

**Saturated Chain Factors as Indices for Correlating the Cold Flow Properties of Biodiesel.** Robert Dunn, USDA ARS NCAUR, USA
The cold flow properties of biodiesel are an ongoing concern for industry and consumers. Development of new feedstocks often yields lipids with high concentrations of high-melting long-chain saturated fatty acids. Conversion of such lipids to fatty acid methyl esters (FAME) results in biodiesel with high cloud point (CP) and cold filter plugging point (CFPP) values. measurement of these properties takes time and often requires expensive instrumentations. This study develops saturated chain factors as indices for correlating the cold flow properties of biodiesel (FAME). Results showed that the saturated chain factor indices may be used to calculate the CP and CFPP of biodiesel within 1.2 °C, with respect to measured properties. These indices and their associated correlation models offer a cost-effective means for determining the cold flow properties and performance of biodiesel derived from diverse lipid feedstocks.

**Strength of Transesterification Catalysts Determined by oxygen-17 NMR.** Sarah Purdy, University of Saskatchewan, Canada; Jianheng Shen, University of Saskatchewan, Canada; Jianfeng Zhu, University of Saskatchewan, SSSC, Canada; Martin Reaney, University of Saskatchewan, Canada
The study of the alkoxide catalyzed biodiesel production is of great interest and previous studies have probed it indirectly by 1H and 13C Nuclear Magnetic Resonance spectroscopy (NMR). The catalysis activity can be directly observed by NMR using the 17O ($I = 5/2$) nuclei in order to determine the optimal catalyst species and concentration. In this work, measurements were performed on natural abundance, neat samples with the spin-echo pulse program using a Bruker Avance III HD 600 (14.09 T) spectrometer operating at 81.3 MHz. The spin-echo pulse program decreases signal intensity, but completely flattens the rolling baseline and therefore significantly increases signal resolution compared to single pulse programs.

The resolution was further improved by increasing the sample temperature (e.g. 295 K for sodium methoxide, 365 K for sodium glyceroxide). Catalyst solutions of metal ion (Li, Na, K), alkoxide species (methoxide, isopropoxide, glyceroxide), alkoxide concentrations (0 - 25% v/v), and solvents (methanol, glycerol) were prepared as transesterification catalysts capable of converting triglycerides to fatty acid methyl esters (FAME). The catalyst solutions, liquid glycerol and FAME products were compared using the 17O-NMR spectra. Since this method is specific to the oxygen atom and the signal area is directly proportional to the concentration of the functional group, it offers an excellent method of quantitative analysis. It can be a very powerful tool for the investigation of oxygen functionalities in the complex mixtures present in synthetic biofuels.

**Utilization of Controlled Flow Cavitation to Enhance Biofuel Processing.** Darren Litle, Arisdyne Systems Inc., USA; Oleg Kozyuk, Arisdyne Systems, Inc., USA; Peter Reimers, Arisdyne Systems, Inc., USA

Biodiesel production begins by reacting triglycerides with an alcohol and a catalyst. The products of this reaction are mainly biodiesel and glycerin. The catalyst will form soap and eventually salt due to neutralization in the downstream process. Controlled Flow Cavitation can be used in the transesterification reaction to create micro droplets which form an emulsion. Precise process control ensures a tightly controlled, repeatable droplet size distribution. Superior mixing and high droplet surface area allow for a fast and efficient reaction, which increases the catalyst efficiency. The upcoming tighter specification is asking for lower monoglyceride contents in the end product biodiesel. The most efficient way to achieve a lower monoglyceride content is to push the reaction with a higher catalyst dosage. Controlled Flow Cavitation offers an opportunity to lower mono- and di- glyceride content with no additional catalyst consumption.

**Variables Controlling the Composition of Precipitates Formed in Commercial Biodiesel Fuels and Collected by Fuel Filters.** Richard Heiden, R W Heiden Associates LLC, USA; Sigurd Schober, University of Graz, Austria; Martin Mittelbach, Universtity of Graz, Austria

Saturated monoglycerides (SMGs), glycerol (GLY), and steryl glucosides (SGs), common systemic impurities found in FAME fuels, form ‘‘soft’’ heterophases (HPs) when concentrations exceed solubility limits. Previously, we have discussed the unique solubility behavior of each. Here, an in-depth examination of field specimens further illuminates clues to the conditions that cause the formation of filter-blocking HPs. For more than a decade concerns about damage to newer HPCR diesel engines from ‘‘hard’’ particles have resulted in the constriction of rated pore dimensions of fuel filters to the low micron range. Though the intent is to remove ‘‘hard’’ particles, filters also increasingly capture the polar HPs that earlier would elude filters and pass right through to injection devices and combustion zones. Fuel filtration obstruction incidents observed globally, most commonly during cold spells, represent the most acute and tangible evidence that common thermodynamic variables controlling solubility in dilute solutions work together to force the formation of collectible HPs. Drawn from over 30 filter obstruction events, chosen filter deposits were accompanied by fuel liquids drawn from vehicular storage tanks containing 8–19% FAME that appear to have met existing specifications. Precipitation of HPs in fuels was induced by cooling to temperatures well above cloud points. Analyses by GC-FID, GC-MS and light microscopy revealed products of interactions or coprecipitation processes. Comparisons of the HPs induced in-situ in the lab with those collected by fuel filters suggests that filtration through commercial devices can lead to compositional separations due to differences in the physicochemical properties of HPs, such as size, likely a reflection of the formation environment. Certain HPs at 0.003%m/m or less in fuel liquid at 20–25C can contribute to appreciable flow obstructions. Other HP formers, such as GLY or SMGs, require cooler temperatures, elevated concentrations, and/or interactions to induce blockage-forming assemblages.

## Industrial Oil Products Poster Session

*Poster Chair Jerry King, CFS, USA*

**Comparing 2-Methyltetrahydrofuran with Hexane for the Lipid Extraction of Fresh Black Soldier Fly Larvae (Hermetia illucens) as Renewable Feedstock for Industry, a Designed Approach.** Ruben Smets, KU Leuven, Belgium; Peter Goos, KU Leuven, Belgium; Johan Claes, KU Leuven, Belgium; Mik Van Der Borght, KU Leuven, Belgium

Black soldier fly larvae (BSFL, Hermetia illucens) have the potential to become a valuable and renewable feedstock for the oil and fats industry due to their high lipid content (18–48 %) and interesting fatty acid profile. Although hexane is a typically used lipid extraction solvent; green solvents are becoming increasingly popular to replace harmful solvents. In this work, the green solvent 2-methyltetrahydrofuran (2-MeTHF) was compared with hexane for extracting lipids from fresh BSFL using Design of Experiments (DoE) with a Soxhlet extraction of dried BSFL as benchmark. By using DoE for optimising the lipid extractions, two sets of optimal process settings for maximising the solvent efficiency and the lipid recovery were predicted and validated experimentally. Additionally, resulting lipid extracts were analysed for their fatty acid profiles and lipid class

compositions. The results of the benchmark extraction showed that 2-MeTHF extracted significantly more lipids than hexane (43.23 vs. 40.34 g lipids/100 g). By performing the wet extraction with 2-MeTHF at 45 °C with 15 w/w % fresh BSFL, 40 w/w % 2-MeTHF, and 45 w/w % water; the highest lipid recovery was obtained (94.9 ± 1.8 %). In terms of actual lipid yield, around 41 g lipids (on dry matter) were extracted, which was highly similar to the results of the benchmark extraction. Concerning the fatty acids, no differences were observed between hexane and 2-MeTHF with all extracts containing high quantities of lauric (± 35 %), oleic (± 20 %), palmitic (± 12%), and linoleic (± 10%) acid. Regarding lipid class distributions, higher amounts of free fatty acids and phospholipids were extracted with 2-MeTHF. Consequently, the results of the present work indicated that the lipid extraction from fresh BSFL with 2-MeTHF can be considered a suitable, more ecologic alternative for the conventional extraction of dried BSFL with hexane.

**Enzymatic editing of vegetable oils to obtain a low diglyceride (DG) oil by two methods—specific hydrolysis of DG and conversion of DG to TG by using a unique lipase.** Tomohiro Kimura, Amano Enzyme Inc., Japan; Tatsuya Shinoda, Amano Enzyme Inc., Japan; Yuzo Kojima, Amano Enzyme Inc., Japan; Shotaro Yamaguchi, Amano Enzyme Inc., Japan

Diglyceride (DG) in vegetable oils is an undesirable substance, lowering the fractionation efficiency and the oil thermostability, and may be one of the factors for glycidol ester formation. There is no good method to reduce DG in fats and oils industry.

We propose the enzymatic methods for production of low DG oil using an unique lipase derived from Penicillium camemberti, Lipase G ''Amano''. Firstly, it can hydrolyze DG and monoglyceride (MG) but not triglyceride (TG), thus, it is possible to decrease DG contents without affecting to TG yield by its hydrolytic action. Immobilized lipase G (Lipase GS-IM) could be used repeatedly.

A second characteristic of Lipase G is that it can synthesize TG from DG and FFA in a microaqueous environment without trans/interesterification of TG. Some lipases, for example Rhizopus lipases, also can synthesize TG from DG and FFA under the microaqueous condition but these lipases bring about trans/interesterification of TG at the same time. Because of no trans/interesterification ability of TG, the Lipase G-treated low DG oil have a characteristics similar to the original oil.

The unique Lipase G will enable the production of value-added low DG oils under an environment friendly condition.

**Hydroboration-oxidation as a tool to access chiral trifunctionalized triglyceride scaffolds.** Francisco Leyva Gutierrez, University of Tennessee Knoxville, USA

Access to hydroxyl-functionalized triglycerides has been limited to epoxidation and epoxide ring-opening reactions. These viscous liquid intermediates find use primarily as plasticizers and raw materials for polyurethanes in addition to paints and cosmetics. Methods for incorporating a single hydroxyl functionality per fatty acyl chain are scarce, however. The hydroboration-oxidation of triglycerides is remarkably facile and results in solid, trifunctionalized intermediates with a single hydroxyl substituent per fatty acid acyl chain, analogous to hydrogenated castor oil. The distribution of 9- and 10-, (R) and (S) hydroxy enantiomers can be determined via 1H NMR spectroscopy with the aid of a chiral shift reagent and has been found to depend on the choice of hydroborating agent and temperature. Borane-tetrahydrofuran complex can provide up to a 70% selectivity for the 9- and 10-(R) enantiomers, likely due to steric implications.

The trifunctionalized triglycerides serve as a useful scaffold to access more exotic ketone and amine functionalized triglycerides. Oxidation to the ketone is essentially quantitative via Dess-Martin conditions. Amines can be obtained in 40% yield via reductive amination of the ketone. Bayer-Villiger oxidation of the ketones provides further access to an internal ester functionality.

The utility of these new trihydroxy-functionalized triglycerides as post-harvest coatings for fresh fruit commodities has been investigated. Incorporating up to 50 wt% of this new material into commercial carnauba wax-based coating formulations was found to match the moisture barrier properties of pure carnauba-based emulsions, representing an economic boon. In addition, the onset of crystallization for carnauba-based emulsions was found to decrease by up 34 °C with inclusion of only 20 wt% trihydroxy-functionalized triglyceride. With a melting point range of 50–52 °C, this new material shows promise as potential emulsion and rheological modifier applicable to the food and cosmetic industries.

**Improvement of dyeing para-aramid fabrics pretreated with soybean oil and nonthermal plasma in glycerol-based dye bath.** Xiaofei Ye, University of Tennessee, USA; Mary Morris, University of Tennessee, USA; Christopher Doona, U.S. Army Combat Capabilities Development Command – Soldier Center, USA

The color of woven para-aramid fabrics is limited to the color of the spun yarn prior to weaving, because it is very difficult to dye para-aramid textiles, limiting practical applications of the high-performance para-aramid materials. This study developed a new method to improve the dyeing color strength by soaking para-aramids in soybean oil followed by surface treatment with atmospheric nonthermal plasma. Sequential experimentation proved the beneficial effects of soybean oil in

dyeing para-aramids combining with nonthermal plasma treatment, the use of glycerol as dispersant, and the addition of sodium chloride as electrolyte without other auxiliary chemical additives. At the optimized condition of these factors, the color strength of dyed para-aramids increased from 0.35 of untreated samples to 3.89 evaluated as K/S values calculated from the spectral analysis of the sample reflectance in the visible range. Fourier Transform Infrared analysis revealed that the high content of unsaturated fatty acids in soybean oil and the interaction with nonthermal plasma, are responsible for the improved dyeing color strength.

**Preparations of gels using cedarwood oil and plant waxes for an insect barrier.** Fred Eller, USDA ARS NCAUR, USA

The purpose of this study was to develop cedarwood oil (CWO) into a convenient formulation that could be used as a barrier to crawling insects. A variety of oleogelators (e.g., plant waxes, bees wax and tristearin) and structuring agents (e.g., silica gel and cellulose) were tested. Oleogel mixtures were compared by placing a drop of the test mixture on the side of a glass beaker and then exposing the beaker to increasing temperatures starting at 25C. The temperature at which the mixture melted and slid down the beaker was recorded. The goal was to resist melting up to a temperature of at least 60C (i.e., 140F). Different concentrations of CWO in oleogelators were tested to attain the highest concentration of CWO possible. In general, higher concentrations of oleogelators led to higher melting points. A CWO mixture containing bees wax did not resist melting as well as a mixture containing tristearin. At concentrations of 10% plant wax (i.e., 90% CWO), rice bran bran wax and sunflower wax gave gels that melted at the highest temperature, while at 15% plant wax (i.e., 85% CWO), carnauba wax melted at the highest temperature. Cedarwood oil formulated with silica gel resisted melting better than CWO formulated with cellulose. Some of these plant waxes are suitable for preparing CWO for use as an insect barrier. Field trials are planned to evaluate their effectiveness.

**Reaction Kinetic Study of Synthesis of Lubricity Improver Additive from Methyl Oleate.** Randy Maglinao, Montana State University-Northern, USA

Fatty acid methyl esters have been proven to be an excellent lubricity improver for ultra-low sulfur diesel. However, it has undesirable cold flow performance and unsatisfactory oxidative stability. In our previous studies, we successfully improved biodiesel's cold flow and oxidative stability properties through alkylation with aromatic compounds. To better understand the reaction mechanism involved in process, we conducted series of experiments to study its reaction kinetics. The alkylation of methyl oleate, a fatty acid methyl ester, and toluene was carried out in a 20-mL batch reactor over a montmorillonite clay catalyst. At the end of the reaction, the products were immediately cooled through a condenser and collected for analysis. The experimental data show that the reaction follows an Eley-Rideal mechanism. Diffusion of the reactants to the catalyst was also found significant when the reactants were not stirred with the catalyst.

**Scaling of sustainable technologies to transform glycerol into added-value energetic compounds.** Raul Comelli, Instituto de Investigaciones en Catálisis y Petroquímica "José Miguel Parera" – INCAPE (UNL, CONICET), Argentina; Diego Garcia Touza, Varteco Quimica Iberica SLU, Spain; Lisandro Ferrari, Varteco Quimica Puntana S.A., Argentina; Fernando Tuler, Instituto de Investigaciones en Catálisis y Petroquímica "José Miguel Parera" – INCAPE (UNL, CONICET), Argentina; Carlos Casas, Varteco Quimica Puntana S.A., Argentina

Glycerol, the co-product in biodiesel production, is intermediary for synthesis of a large number of compounds for industry. In Argentina, in the Province of Santa Fe, a capacity of 4.0 Mtons annual of biodiesel is installed, being available 400,000 tons/year of glycerol. The problem of its increasing availability is not resolved adopting a monoproduct strategy, because this one would become the new problem; consequently, solution must have a diversification approach and the concept of Biorefinery can be applied. Since 2007, catalytic processes have been developed to convert glycerol into industrially applicable compounds with high added-value, such as dihydroxyacetone (DHA), acetol, propylene glycol (PG), ethylene glycol (EG), 1,3-propanediol, lactic acid, and methanol, as well as compounds with energetic use such as hydrogen, syngas, and ethers of glycerol. Reactions of selective oxidation and reduction, hydrogenolysis, and steam reforming were studied, showing the possible integration of processes in a biorefinery environment; DHA, acetol, PG, EG, lactic acid, hydrogen, syngas, carbon dioxide, methane, and methanol can be obtained feeding only glycerol. Three catalysts and processes were developed and patented: i) selective oxidation to DHA on Pt-Bi/K-FER, reaching 75.9% glycerol conversion and 93.9% selectivity to DHA; ii) selective reduction to PG in gas phase on Cu-containing catalysts, reaching the best performance Cu-Ce/Alumina, with 99.8% glycerol conversion and 83.2% and 14.3% selectivities to PG and acetol (the reaction intermediate), respectively; and iii) hydrogenolysis to EG on Ni/SiO2, obtaining 100% glycerol conversion and 91.0% selectivity to EG in the liquid fraction. Finally, from a strong link with the productive sector, financing was obtained to build two pilot plants, one to produce 100 tn/year of PG but versatile to also produce acetol and/or EG, and another one for reforming glycerol to obtain hydrogen, which is raw material for selective reductions; both plants would be in operation during 2021.

**Separation of Fusel Oils by Distillation.** Farley Chicilo, University of Saskatchewan, Canada; Timothy Tse, University of Saskatchewan, Canada; Sarah Purdy, University of Saskatchewan, Canada; Josseline Ramos-, Figueroa, University of Saskatchewan, Canada; Jianheng Shen, University of Saskatchewan, Canada; Chris Zhang, University of Saskatchewan, Canada; Ramaswami Sammynaiken, University of Saskatchewan, USA; <–**s/b Canada?*** Martin Reaney, University of Saskatchewan, Canada

As a result of the Covid-19 pandemic, biofuel ethanol production has become an important source of higher grades of ethanol used in producing disinfectants and hand sanitizers like United States Pharmacopeia (USP) grade. While fusel oil can be included in biofuel, the mixture of higher alcohols, including amyl, n-propyl, and isobutyl alcohols are only allowed in small concentrations in USP ethanol. Fusel oil compounds accumulate in yeast fermented solutions through the action of the Ehrlich pathway. During fractional distillation they can accumulate in the rectifier and are removed in a fraction that contains ethanol and water to improve distillation performance. The resulting fusel oil/ethanol/water mixture can be further distilled, separating recovered ethanol from fusel oils. Fusel oils can be recovered for industrial applications, burned to supply energy for a plant, or be used in fuel additives [[i]]. In this project, the components of a mixture of fusel oils procured from local and sustainable sources, are performed using multiple high resolution distillation techniques (ex: fractional, spinning band distillation) in both laboratory and larger scale applications. The distilled materials obtained using these various techniques are compared using gas chromatography, and the efficacy of each method is compared. Using high resolution distillation techniques, technical grade ethanol is produced, and has the potential to be used as a disinfectant for hand sanitizer applications. Additionally, this process separates the higher alcohols present in fusel oil, allowing for the materials to be repurposed and reducing the cost for manufacturers.

[i] Patil, A. G.; Koolwal, S. M.; Butala, H. D. Fusel oil: Composition, removal and potential utilization. Int. Sugar J. 2002, 104, 51.

## Lipid Oxidation and Quality

*Division Vice Chair: Karen Schaich, Rutgers University, USA*

## Actions of Catalysts and Antioxidants in Lipid Oxidation

*Chairs Lan Ban, Kemin Food Technologies, USA; Karen Schaich, Rutgers University, USA*

**Characterizing Endogenous Pro-oxidants and Potent Antioxidants in Muscle Food Systems.** Mark Richards, University of Wisconsin, Madison, USA; Haizhou Wu, Chalmers University of Technology, Sweden; Nantawat Tatiyabororntham, National Center for Genetic Engineering and Biotechnology, Thailand

There are many possible pro-oxidants in muscle foods that include low molecular metal complexes, lipoxygenases, myoglobin, and hemoglobin. An inhibitor selective for hemoglobin was shown to strongly inhibit lipid oxidation in comminuted trout whole muscle, turkey thigh muscle, and salted pork semimembranosus. This suggested residual hemoglobin was the major pro-oxidant in these muscle foods in a highly comminuted state. The ability of hypervalent hemoglobin species and heme released from the globin represent two possible pathways by which hemoglobin can promote lipid oxidation. The heme release mechanism was investigated by using site-directed mutagenesis of sperm whale myoglobin to produce variants with low and high heme affinity. The low heme affinity variant readily facilitated lipid oxidation in washed muscle while the high heme affinity variant was weakly pro-oxidative. These finding implicated heme release as a likely mechanism by which hemoglobin promotes lipid oxidation. Regarding antioxidants, we have found that quercetin was more effective at inhibiting lipid oxidation in muscle systems compared to glycosylated quercetin indicating increasing water solubility of the antioxidant decreased efficacy. Further, propyl gallate most effectively inhibited lipid oxidation compared to gallic acid, octyl gallate, and lauryl gallate, indicating intermediate polarity most effectively inhibited lipid oxidation. We have also shown that phospholipase A2 (PLA2) can be highly effective at inhibiting hemoglobin-mediated lipid oxidation. We observed a lipid hydroperoxide depletion mechanism by PLA2 without formation of secondary lipid oxidation products. It is postulated that cysteine or methionine residues of proteins in muscle reduce fatty acid hydroperoxides released from phospholipids by PLA2 to fatty acid hydroxides that are weakly reactive. Other antioxidant mechanisms of PLA2 may involve rearrangement of the lipid bilayer or antioxidant effects of the hydrolysis products, namely lysophospholipids and free fatty acids.

**Copper-water Interactions in Fish Meal: Impact on Hydroperoxide Formation.** Carrie Wray, Kemin Nutrisurance Inc., USA; John Sander, Kemin Nutrisurance Inc., USA

Copper content varies greatly among commercially produced fish meals. Metal ions may be deactivated via strong coordination with endogenous components in complex matrices (e.g., fish meal); however, even trace amounts of redox-active metals initiate free radical reactions. Literature shows the true impact of transition metals on lipid oxidation, in particular on polyunsaturated lipids, can be obfuscated using the peroxide value (PV) method given metal ions also catalyze the

decomposition of lipid hydroperoxides to volatile species.

Exploratory salmon meal studies showed inconsistent PV accumulation over time. Investigations into potential contributing factors revealed copper exhibited a strong influence on PVs, but with inconsistent trends. Copper addition lead to increased or decreased PVs or had no effect on PVs. All studies used an equivalent copper spike to salmon meals of varying moistures, suggesting a possible copper-water interaction.

Considering the fish meal industry relies on PVs to indicate meal quality, a complete understanding of that value is crucial. An experiment was designed to manipulate the moisture and water activity of fish meal stabilized with tocopherols. For each moisture level, PVs were measured in meal with endogenous and spiked copper concentrations. Additionally, volatile compounds associated with the lipid oxidation of fish matrices were measured via GC-MS to provide an expanded understanding of the oxidative degradation.

Results showed that increased moisture in fish meal decreased PVs. Increased PVs were evident for copper-spiked meal; however, the increase in PVs from copper spiking was lessened at higher moistures. Contrary to PVs, volatile compound accumulations showed an increase with moisture and copper. The measurement of volatile compounds suggests a degree of fish meal oxidation not captured by the PV method and provides further information on the quality of fish meal.

**How the Fatty Acyl Peroxyl Radical Determines the Outcome of Lipid Peroxidation.** Claus Schneider, Vanderbilt University, USA

The initial event in lipid peroxidation is a hydrogen abstraction from an allylic or, preferentially, bis-allylic methylene unit of a (poly-)unsaturated fatty acid. This is followed by addition of molecular oxygen to the fatty acyl radical that is delocalized over three or five carbons, depending on the oxidizing fatty acid. Addition of molecular oxygen to the carbon radical is diffusion-controlled, i.e., it occurs exceedingly fast with essentially no energy barrier. The resulting peroxyl radical, in contrast, has a considerable life-time: therefore, it can undergo a number of different reactions, and which one it ''chooses'' depends on the reaction conditions and the presence of other reagents like fatty acid ''substrates'' and antioxidants in the reaction mixture. The presentation will provide an overview of the reactions of the peroxyl radical, including the formation of the unusual bis-allylic peroxyl radical and how the corresponding bis-allylic hydroperoxide can be formed and stabilized.

**Modulating Interfacial Concentrations of Antioxidants in Oil-in-water Emulsions to Control Antioxidant Efficiencies.** Carlos Bravo-Diaz, University of Vigo, Spain; Marlene Costa, Universidade do Porto, Portugal; Sonia Losada-Barreiro, Universidad de Vigo, Spain; Fátima Paiva-Martins, Universidade do Porto; Portugal

The inhibition of lipid oxidation by employing antioxidants (AOs) is challenging because basic aspects of the reactions involved are still far from being completely understood. Kinetic aspects of the reactions involved have been frequently overlooked, the site of reaction is often uncertain and the knowledge on the effective concentrations of reactants in the different regions of the emulsion has been frequently ignored.

Here we report on an on-going investigation on the control of lipid oxidation by antioxidants. We determined both the oxidative kinetic profiles and the effective concentrations of the antioxidants in the same intact oil-in-water emulsions. Results provide physical evidence for the interfacial region as the main reaction site between AOs and peroxyl radicals, indicating that AOs accumulate in the interfacial region, where their effective concentration is 20–180 times higher than the stoichiometric concentration, depending on the particular surfactant concentration employed in the preparation of the emulsion. Results show that direct relationships between interfacial concentrations and oxidative stabilities can be established. Thus, antioxidant efficiency in oil-in-water emulsions can be improved by targeting antioxidants to interfaces.

**The Multiple Protective Role of Phenolic Compounds in Food Products.** Rosario Zamora, CSIC-Instituto de la Grasa Spain; Francisco J. Hidalgo, CSIC-Instituto de la Grasa, Spain

Antioxidant/prooxidant properties of phenolic compounds are still poorly understood. This is likely due to both the variability of structures of phenolic compounds and the diverse mechanisms by which they can either inhibit lipid oxidation or limit its consequences. The objective of this paper is the study of the structure-activity relationship of phenolic compounds for the inhibition of reactions in which either free radicals or reactive carbonyls are involved. Thus, the mechanisms responsible for the inhibition of lipid oxidation consequences by phenolic compounds will be analyzed and discussed at the molecular level as a function of phenolic structure. Obtained results demonstrate that phenolic compounds do not carry out all protective functions. In fact, phenolics should be classified according to the exhibited functions, which are a consequence of their structure. This classification would allow the selection of the most appropriate phenolic compounds for a specific function, and it would both limit the amount of needed phenolics and avoid the sometimes-observed prooxidant effects.

**Transition Metals Shift Products as Well as Alter Rates in Catalysis of Lipid Oxidation.** Karen Schaich, Rutgers University,

Even though metals are widely recognized as the most ubiquitous catalysts of lipid oxidation in oils and foods,

the mechanisms by which metals catalyze lipid oxidation remain poorly understood, especially in terms of effects on oxidation pathways and product distributions, and development of potentially toxic compounds. With current reformulation of foods to include essential polyunsaturated fatty acids, concern about oxidation has again come to the forefront. At the same time there is increasing recognition that lipids oxidize by multiple pathways. Hence, now is an opportune time to revisit the role of metals in lipid oxidation, examining their multiple catalytic mechanisms and how they may shift lipid oxidation pathways and products as well as increase oxidation rates. This paper presents an overview of mechanisms by which metals catalyze lipid oxidation, with emphasis on important changes in product distributions mediated by metals in addition to effects on oxidation kinetics. These altered oxidation patterns will then be connected to effects on food quality for consumers and to new challenges to analytical capabilities in quality control and research. Important aspects of metal catalysis of lipid oxidation needing up-to-date research will be identified.

## Antioxidant Applications

*Chairs David Johnson, Kalsec Inc., USA; Liyun Ye, Finless Foods, Inc., USA*

**Antioxidant Activity of Peptides Embedded in Potato, Seaweed, Rubisco and Single Cell Proteins.** Betül Yesiltas, Technical University of Denmark, Denmark; Pedro J. García-Moreno, University of Granada (UGR), Spain; Egon B. Hansen, Technical University of Denmark, Denmark; Paolo Marcatili, Technical University of Denmark, Denmark; Tobias H. Olsen, Technical University of Denmark, Denmark; Simon Gregersen, Aalborg University, Denmark; Charlotte Jacobsen, Technical University of Denmark, Denmark

Sustainable eating habits have increased the demand for alternative sources of functional and natural ingredients such as plant and microbial based peptides with antioxidant activity. For some of these sources (potato, seaweed, rubisco and single cell proteins), we have used bioinformatics and proteomics to identify and predict embedded peptides with good radical scavenging and metal chelating activities. Proteomics was also used to confirm that selected peptides are abundant in their parent proteins. The objectives of this study were (i) to screen in-vitro antioxidant activity of synthetic peptides that are predicted to be good antioxidants and (ii) to evaluate the ability of these peptides to retard lipid oxidation in 5% fish oil-in-water emulsions. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured to evaluate the in vitro antioxidant efficacy to scavenge free radicals. It was found that some of the peptides were indeed good radical scavengers (IC50: 2–6 mM) as predicted. Ferrozin method was used for metal chelating activity; however, solubility issues resulted in unreliable results. Based on the highest in vitro radical scavenging activity, bioinformatics prediction, abundancy of peptides in the parent protein and their potential for being released by trypsin, 11 peptides were selected for further validation of their ability to prevent lipid oxidation in 5% fish oil-in-water emulsions. Iron-mediated emulsions were produced using 1 wt.% Tween 20 and 0.05 wt.% synthetic peptides at pH 7 and the oxidative stability was assessed by determining the formation of primary oxidation products, the consumption tocopherols, and the development of volatile oxidation products during 8 days of storage. Additionally, physical stability was also investigated with zeta potential and droplet size distribution analyses. Results showed that peptides from all sources improved the oxidative stability of the emulsions compared to the control, without changing the physical stability largely.

**Determination of Lipid Hydroperoxides in Oil-in-water Emulsions Using by a Novel Technique.** Sibel Uluata, Inonu University, Turkey; Gokhan Durmaz, Inonu University, Turkey; David McClements, University of Massachusetts Amherst, USA; Eric Decker, University of Massachusetts, Amherst, USA

Krill oil has been accepted as a new source omega-3 fatty acids and these fatty acids have many health benefits. On the other hand, nanoemulsions have some important potential advantages over conventional emulsions due to the high physical stability, and ability to increase the bioavailability of lipophilic bioactive. Conventional lipid hydroperoxides methods (titrimetric, AOCS, thiocyanate) did not work to determine lipid hydroperoxides in this emulsion system. DPPP itself is not fluorescent, but when it reacts with lipid hydroperoxides, its oxide form was generated, and it has fluorescent properties. In this study, DPPP (diphenyl-1-pyrenylphosphine) was used a fluorescent probe to determine lipid hydroperoxide by a novel technique. Krill oil-in-water emulsions were prepared using by microfluidizer at 12000 psi pressure. The mean particle diameter was determined around 179 nm and the droplet charge (ζ-potential) was determined from –29 mV. Emulsion samples were incubated at 37oC photooxidation. Lipid hydroperoxides were determined by fluorescens spectrophotometer using with DPPP probe. The results show that fluorescent technique can be used to determine lipid hydroperoxides at different environmental conditions and also this technique was more sensitive and selectivity than conventional techniques.

**Development of an Effective Dipping Strategy to Prevent Lipid Oxidation of Herring (clupea Harengus) Filleting Co-products.** Haizhou Wu, Chalmers University of Technology, Sweden; Ingrid Undeland, Chalmers University of Technology, Sweden

The demand for high value proteins is increasing every year due e.g., to population growth and a diet shift to more proteins driven e.g., by an aging population. Co-products emerging in fish filleting operations contain significant amounts of muscle rich in high value protein, which could be better used in food production to cover the growing protein demand. However, applying value-adding techniques to fish filleting co-products is rendered difficult due to their high susceptibility to lipid oxidation. In the first part of this study, seven components/formulas (water-soluble rosemary extract (RE-A), oil-soluble rosemary extract (RE-B), isoascorbic acid (IAA), esculetin, α-tocopherol (α-TOC) or and two rosemary-containing commercial mixtures; Duralox MANC-213 (MANC) and EVESA-C5 (EVESA)) were directly added into minced herring co-products to screen their ability to prevent lipid oxidation during ice storage. The order by which the seven compounds delayed lipid oxidation was as follows: RE-B (0.05%) = MANC (0.5%) = EVESA (0.5%) > IAA (0.5%) > RE-A (0.2%) > esculetin (0.09%) > α-TOC (0.02%). The two most promising candidates based on both cost and efficiency were used in a second trial where herring co-products were dipped in antioxidant-containing solutions prior to subsequent cold-storage for 12 days in a minced or intact form. The effect of re-cycling the antioxidant solutions was also studied, and both PV and TBARS were analyzed. Dipping of the co-products with a 0.2% RE-B solution with and without 0.5% IAA added remarkably increased the oxidation lag phase from < 1 day to >12 days during subsequent cold storage in minced or intact form. Even after re-use of the dipping solution up to 10 times, lipid oxidation was completely inhibited. The presented dipping strategies could hereby facilitate more diversified end-use of herring co-products from current fish meal to high-quality minces, protein isolates or oils for the food industry.

**Insights of Impact of Acetic Acid on Antioxidant Activities of Natural Phenolic Compounds at Low pH Range in Tween 20 O/W Emulsion and Mayonnaise.** Hefei Zhao, University of Nebraska-Lincoln; University of California-Davis, USA; Changmou Xu, University of Nebraska-Lincoln, USA Clean-label is the current trend in the food industry. Many studies have been conducted to test the antioxidant possibility of natural phenolic compounds (PC) in mayonnaise, however, their performances were intrinsically weaker than EDTA, or even showed prooxidant effect.

To understand the antioxidant mechanism at low pH, the performance of natural antioxidants from previous studies were summarized. Then chelation ability, DPPH, and ferrous reducing antioxidant power (FRAP) were determined at pH6.8 and 3.9. Oxidation experiments were implemented in both Tween20 O/W emulsion and mayonnaise.

EDTA maintains chelation at both pHs, but those of PC, polyacids drastically reduced at pH3.9. EDTA and polyacids did not have electron donation, while PC has electron donation ability at both pHs. The effects of EDTA, phytic acid(PA), and natural phenolic compounds(NPC) including quercetin(Q) tannic acid(TA) and grape skin extract(GKE) on the oxidative stability of Tween 20 O/W emulsion and mayonnaise were evaluated. EDTA 70ppm, and 300ppm of PA, TA strongly inhibited more than 88.70% TBARS in Tween20 O/W emulsion with 4.214 ppm iron due to their chelation at pH6.8. At pH3.9, EDTA and PA inhibited more than 91.55% of the oxidation in Tween 20 O/W, but the TA only inhibited 40.62% of the oxidation. However, GKE 300ppm performed a prooxidant effect due to iron reduction to promote Fenton reactions. Also, under acidic conditions in mayonnaise, although EDTA still strongly inhibit 79.01% of lipid oxidation, and other natural compound showed inhibition of lipoid oxidation by 27.60% of Quercetin 300ppm, 43.78% of Quercetin 600ppm, 25.91% of Tannic acid 300ppm, 59.16% of Tannic acid 600ppm, the phytic acid performed little antioxidant or even prooxidant effect possibly due to its charge interaction with proteins.

This study reveals that the pH and protein in the food matrix critically influence the antioxidant performance of natural compounds in actual fatty foods.

**Model System to Screening Plant Extracts for EDTA Replacement for Lipid Emulsions.** Drew Elder, Kalsec Inc., USA; Cindy Tian, Kalsec Inc., USA

Stabilizing food emulsions has always been a challenge for food manufacturers. Under today's clean-label environment there's a high demand for natural antioxidant products with enhanced performance to stabilize such products. To tackle this challenge, an unique stageous approach was used to evaluate antioxidant properties of plant extracts and to identify species and combinations that deliver high level of activity for different food applications. As part of the approach, a ranch dressing model was developed to study natural antioxidants for lipid emulsions. A polyphenol-rich plant extract from the Lythraceae family and its different combination with rosemary extract was found to be highly effective in such model. Their combinations were optimized and then tested against the synthetic additive Ethylenediaminetetraacetic acid (EDTA). This natural antioxidant blend provided equal effectiveness in inhibiting generation of oxidation products and in minimizing sensorial changes of the ranch over time. The antioxidant mechanism of such combination in low-pH systems was explored and studied in vitro. Conclusion from this research will be useful in guiding the application of natural antioxidants to achieve optimum performance in lipid emulsion applications.

## Industry Updates II

*Chair Larry Tucker, Metrohm USA, USA*

**Oxidative Stability of Edible Oils: An Indicator of Quality.** Kerri-Ann Blake, Metrohm USA, USA
The quality and value of edible oils are linked to oxidative stability, i.e., rancidity, and shelf life. Oxidative degradation of oils will occur naturally over time but oils that remain stable longer will command a higher selling price. Some oils contain natural antioxidants which extend their lifespan while others require the addition of antioxidants to remain competitive. Therefore, oxidative stability is an important parameter to determine in edible oils.
While determining acid value and peroxide value by titration can be a good indicator of rancidity, these are not predictive tests and cannot establish the time span until spoilage. In contrast, the Rancimat method predicts product usability and shelf life by accelerating aging. During analysis, oils are placed in a closed reaction vessel at a constant temperature with air passing through the sample. Oxidation products are then measured to establish the oxidative stability of the sample. Attend this presentation to learn more about determining the oxidative stability of edible oils and cosmetics using the Rancimat method.

## Lipid Oxidation and Quality Division Student ePoster Pitch Competition

*Judges Charlotte Jacobsen, Technical University of Denmark, Denmark; David Johnson, Kalsec Inc., USA; Nora Yang, Kalsec Inc., USA*

**Antioxidant Activities and Chemical Profiles of Aqueous and Ethanolic Extracts from Heated Sesame Meals in Oil-in-Water Emulsion.** HeeBin Seo, Sungkyunkwan University, Republic of Korea; SoYoon Park, Sungkyunkwan University, Republic of Korea; KeunCheol Yoo, Sungkyunkwan University, Republic of Korea; JiHee Hong, Sungkyunkwan University, Republic of Korea; Seung-Ok Yang, Seoul National University, Republic of Korea; JaeHwan Lee, Sungkyunkwan University, Republic of Korea
Sesame meal is a byproduct of sesame oil processing and contains high percentage of protein, lignans and sesamol. Therefore, many researches have been conducted to utilize sesame meal to get value-added biomaterials. However, studies using oil-in-water (O/W) emulsions as matrix are limited in the literature, although O/W emulsion is one of major food matrices with unsaturated fatty acids.
The objectives of this study were to determine chemical profiles of heated sesame meal and to evaluate antioxidant properties of extracts from heated sesame meal in O/W emulsion.
Comprehensive chemical profiles of extracts were conducted using GC/MS after derivatization. Sesame meal was heated at 0, 140, and 170°C for 1 h and the aqueous and 70% ethanol extracts were obtained. O/W emulsion containing 100 ppm extracts were stored at 50 °C for 2 weeks and degree of oxidation were evaluated.
β-Gentiobiose and sucrose were two dominant chemicals in aqueous and ethanolic extracts. Generally, heat treatment decreased the contents of amino acids, organic acids, carbohydrates and its derivatives. As heating temperature increased, sesamol concentration increased while sesamolin and sesamin decreased in both sesame meal extracts. As temperature of heat treatment increased, oxidative stability of O/W emulsions containing both extracts increased at 50 °C storage.
Sesame meal extracts received thermal treatment possessed higher antioxidant properties in O/W emulsion partly due to the generation of sesamol. Therefore, sesame meal extracts are suitable in enhancing self-life of O/W emulsion based food products as natural ingredients.

**Chemical characterization of Echium plantagineum seed oil obtained by three methods of extraction.** Giovanna Carlini, 1LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Inar Castro, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Gabriela Roschel, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Roseli Ferrari, Institute of Food Technology (ITAL), Campinas, SP, Brazil, Brazil; Severino Alencar, Department of Agri-Food Industry, Food & Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo; Brazil Helton, Ota, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Tayse Ferreira, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, USA
Echium seeds oil has been considered an important alternative to marine oils as source of omega 3 fatty acids, due to the high proportion of stearidonic acid (C18:4 n3). The process applied on the seeds can affect chemical, physical, functional and sensory characteristics of the extracted oil. Thus, our aim was to determine the chemical and sensory parameters of the oil obtained from Echium plantagenium seeds submitted to three extraction techniques: continuous screw press (PRESS), hydraulic press (HYD), and hexane extraction. In addition, a mixture (BLEND) was

prepared containing 70% PRESS oil and 30% of the oil extracted with hexane. Partial results showed that peroxide value (mmol Kg-1 oil) increased from PRESS (0.61 ± 0.01) and HYD (0.86 ± 0.01) to BLEND (1.69 ± 0.01) samples, but were below the Codex Standard limit for unrefined oils (7.5 mmol Kg-1 oil). HYD oil presented the highest concentration of malondialdehyde (351.97 ± 8.40 μmol Kg-1 oil) compared to PRESS (173.13 ± 12.93 μmol Kg-1 oil) and HYD (173.76 ± 4.70 μmol Kg-1 oil) oils. Gamma-tocopherol was found in the higher amount in the PRESS oil (779.21 ± 1.46 mg/Kg oil). In terms of phenolic compounds, rosmarinic, gallic and caffeic acids, as aglycones or glycosides derivatives were found in a higher frequency in the samples, according to the qualitative LC-ESI-QTOF-MS analysis. Although chemical differences have been observed, sensory characteristics were similar among the oils, that presented a general acceptability score of 5.95 ± 0.14 in a scale from 1 to 9, being the green color detected as a negative aspect. Plant sterols and volatile compounds profile will be determined in the next step by GC/MS, providing a better chemical characterization and oxidative stability approach of this important vegetable alternative to fish oil consumption.

**Effect of feeding olive pomace acid oil on lipid composition and oxidative stability of chicken and pork meat.** Paula Albendea, Universitat De Barcelona, Spain; Alba Tres, Universitat de Barcelona, Spain; Gerard Verge-Merida, Universitat Autonoma de Barcelona, USA; Ana Cristina Barroeta, Universitat Autonoma de Barcelona, Spain; Marçal Verdú, BonÀrea Agrupa, Guissona, Spain; Stefania Vichi, Universitat de Barcelona, Spain; Magdalena Rafecas, Universitat de Barcelona, Spain; Francesc Guardiola, Universitat de Barcelona, Spain

Acid oils (AO) are fat by-products from edible oil refining, characterized by their high proportion of free fatty acids (FA). For years, there has been an interest in using them in animal feeding because of their high energetic value.

The aim of this study was to evaluate if the inclusion of olive pomace AO in feeds had any significant effect on FA composition, tocopherol and tocotrienol (T and T3) profile and TBA values in chicken and pork meat.

Broilers (Ross 308) and pigs [(Landrace x Large white) x Duroc] were fed the growing-finishing period (18 and 61 days, respectively) 3 dietary treatments including 6% of palm oil (P), olive pomace oil (OP) or olive pomace acid oil (OPA). After slaughtering, samples of meat with skin from deboned legs of broilers and samples of longissimus dorsi muscle of pigs were taken (8 analytical replicates per treatment in both cases).

FA composition, tocol (T and T3) profile and TBA value were determined in fresh and refrigerated meats under commercial conditions: O2/CO2; 70/30; 3–4°C; 7 days for poultry and 8 days for pork.

FA composition of pork and chicken meat was influenced by the dietary treatment. Tocol profile and total tocol level depended on the type of meat and the dietary treatment. Total tocol concentration was higher in chicken than in pork. In chicken, the highest tocol level was found in OPA and P samples, while in pork the highest was in P and the lowest in OPA meats. Refrigeration storage decreased total tocol levels in pork but not in chicken. Dietary treatment and type of meat influenced TBA values, which showed higher levels after the refrigeration.

Studying the use of AO in animal feeding can contribute to the sustainability of the food chain.

**Evaluation of Oxidative Stability of Full-fat Soybean Flour Stored Under Accelerated Conditions as Influenced by Traditional Processing Methods.** Ece Gulkirpik, University of Illinois at Urbana Champaign, USA; Marco Toc, University of Illinois at Urbana Champaign, USA; Richard A. Atuna, University for Development Studies, Ghana; Francis K. Amagloh, University for Development Studies, Ghana; Juan E. Andrade Laborde, University of Florida, USA

Justification: In Sub-Saharan Africa, full-fat soybean flour (FFSF) is a commercially used, low-cost ingredient to complement the protein content of cereal-based blends. Its high polyunsaturated oil content is prone to oxidation in most tropical climates, however, limiting its use in food applications.

Objective. To examine the effect of pretreatment of soybeans using malting (80%, form hypocotyl), soaking (18h and 24h), roasting (110–120oC, 10 min) and dehulling, on the oxidative stability of FFSF for 12 weeks under room (23 ± 0.1oC, 25.7 ± 5.4% relative humidity, RH) and accelerated conditions (45 ± 0.1oC, 80–82% RH).

Methods: Two batches of FFSF samples were prepared in Ghana one of which was dehulled. Raw samples were the control. Samples (25g) were stored in flat polypropylene heat-sealed bags before placing under either condition. Samples were collected after 0, 4, 8, 12 weeks. Acid value (AV), peroxide value (PV), p-anisidine value (pAV), lipoxygenase activity (LOX), color, moisture, and water activity were measured using standard methods. A three-way Mixed ANOVA was used to evaluate the effect of pretreatment, condition, and time on physical-chemical parameters.

Results: Pretreatment, condition*time, and pretreatment*condition*time had a significant impact on AV, PV, pAV, LOX, color values of all samples ($p < 0.001$). Accelerated conditions promoted oxidation in FFSF regardless of the pretreatment method. Roasted dehulled treatment resulted in the highest increase in AV and pAV in both storage conditions ($p < 0.05$). Raw samples had the highest LOX activity while roasted and malted ones had the lowest. Under accelerated conditions, LOX activity was almost absent in all samples by

the end of 12 weeks. Malting and soaking processing methods significantly reduced oxidation ($p < 0.05$) of FFSF.
Significance: Traditional processing methods such as malting and soaking can improve oxidative stability and therefore potentially expand the shelf life of FFSF. Roasting promotes oxidation despite its common use.

**Exposure of squalene hydroperoxides to photons causes the formation of unique squalene cyclic peroxides via a chain reaction mechanism detected on the human skin and causing harm to skin cells (HaCaT).** Saoussane Khalifa, Tohoku University, Japan; Shunji Kato, Tohoku University, Japan; Masaru Enomoto, Tohoku University, USA; Yurika Otoki, Tohoku University, Japan; Takahiro Eitsuka, Tohoku University, USA; Kiyotaka Nakagawa, Tohoku University, USA
Squalene (SQ) is one of the most abundant skin lipids that plays an overall protective role on the skin. However, it has been demonstrated that SQ on the skin can be readily oxidized to form six monohydroperoxide isomers (SQ-OOH) as the primary oxidation products upon reaction with singlet oxygen. As hydroperoxides are considered to be rather unstable, the secondary oxidation products deriving from their further modification under oxidative conditions are still unclear.
In this study, we focused on the structural elucidation of unique squalene cyclic peroxides deriving from SQ-OOH isomers, the mechanism involved in their formation, their detection on the human skin, and the damage that they can cause to skin cells (HaCaT) in comparison to SQ-OOH.
SQ-OOH isomers were synthesized by singlet oxygen oxidation then purified via multiple steps. The secondary oxidation products were synthesized by subjecting SQ-OOH isomers to a second oxidation, the mechanism generating these compounds was studied by carrying out the second oxidation in different conditions. Their structures were studied using LC-MS/MS, NMR and derivatization reactions. Human skin surface lipids were sampled using acetone-soaked cotton swabs, further extracted and purified, then analyzed using LC-MS/MS. HaCaT cells were used to assess the effect of the primary and secondary oxidation products on the skin.
The secondary oxidation products in question were revealed to be cyclic peroxides deriving from individual SQ-OOH isomers upon their exposure to photons in the presence of ground state molecular oxygen. The presence of the cyclic peroxides derived from 6-OOH-SQ and 10-OOH-SQ was confirmed on the skin, they were also detected in the medium after incubation of 6-OOH-SQ and 10-OOH-SQ with HaCaT cells. This was also linked to a high cytotoxic effect observed with 6-OOH-SQ, 10-OOH-SQ, and even higher in the case where the pure secondary oxidation product deriving from 6-OOH-SQ was used.

**Lipase-Assisted Production of 1-o-galloylglycerol: Characterization and its Antioxidant Properties.** Siyu Zhang, University of Georgia, USA; Casimir Akoh, University of Georgia, USA
1–Galloylglycerol (GG) is a water-soluble derivative of gallic acid (GA), and a phenolic secondary metabolite of many plants. Conventionally, GG is synthesized by chemical esterifications and transesterifications with low yield and the reaction conditions are not suitable for producing bioactive compounds to be used in foods. In this work, GG was synthesized by lipase-assisted glycerolysis of propyl gallate (PG) using an immobilized commercially available food-grade *Candida antarctica* lipase B (Lipozyme® 435). The reaction was performed in a 100-mL double-layer jacketed glass reactor equipped with a circulating water bath, stirred with a PTFE anchor paddle stirring rod, and using glycerol as both the reactant and solvent. Under optimal conditions (55 °C, 25:1 glycerol:PG substrate molar ratio, 120 h, and 23.8% enzyme load relative to the total weight of the substrates) a yield of 76.9% ± 1.2% was obtained. The antioxidant properties, water solubility, n-octanol/water partition coefficient, and molar extinction coefficients of GG were determined after being separated from the reaction mixture using liquid-liquid extraction. The water solubility and hydrophilicity (measured by n-octanol/water partition coefficient) of GG were significantly higher than those of GA and PG. The antioxidant properties, measured by the ferric reducing antioxidant power (FRAP) and hydrogen peroxide ($H_2O_2$) scavenging assays, showed that GG exhibited the highest scavenging capacity (GG > GA > PG). From the results of the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) assays, GG and GA exhibited greater scavenging capacity than PG (GG = GA > PG). GG may be used as a water-soluble antioxidant in food and cosmetic applications.

## Lipid Oxidation and Quality in Novel Alternative Protein Products

*Chairs Karen Schaich, Rutgers University, USA; Liyun Ye, Finless Foods, Inc., USA*

**Cell-based Lean Fish Should Be an R&D Focus to Help Support the Future Demand for Animal Protein.** Greg Potter, Lipiferm Scientific, Canada; Alain Rostain, Clean Research, USA
In the cell-based seafood space several companies have revealed that they are targeting high-value fish including Atlantic bluefin tuna and a species of salmon.

In early 2018, Clean Research identified uncharted territory in cell-based seafood - lean fish - a set of species with 2% or less muscle fat. In this presentation, we make the case in favor of lean fish over other mass-consumed animals for cell-based animal protein production.

To do this, we lead with definitive general information on advantageous aspects of fish cell culture derived from reported experimental findings. These qualities of fish cells relative to mammalian and avian cells include but are not limited to reduced susceptibility to senescence, increased karyotypic stability, and suitability to growth in atmospheric air. Thereafter, we present potential upsides of cell-based fish for research and industrial-scale production, which focus on cell temperature growth range, oxygen requirements and their respective implications on bioprocess design.

We then emphasize further compelling reasons to pursue lean fish specifically, which primarily store lipids in their liver, versus fatty fish, which predominantly store lipids in muscle tissue. These theoretical advantages relate to the technical challenges of co-culturing muscle and fat cells, and the less complex (and more interchangeable) flavour profiles of lean fish compared to fatty fish species broadly. We also detail the fact that lean fish consumption has been shown to confer comparable health benefits to omega-3-rich fatty fish regarding risk factors for cardiovascular disease and type 2 diabetes. Finally, we discuss critical information on the amenability of lean fish for in vitro tissue growth on a scaffold due to the repeated patterning of the muscle tissue and the absence of high levels of adipocytes, compared to the more complex and fat cell-laden structure of a fatty fish filet and a beef steak.

**Impact of Natural Antioxidants on Meat vs. Plant-based Meat Alternatives.** Ajita Sundarram, Kalsec Inc., USA; David Johnson, Kalsec Inc., USA; Cindy Tian, Kalsec Inc., USA; Poulson Joseph, Kalsec Inc., USA

The shelf life of meat products, especially cooked meat products, is limited due to their oxidative stability. In the meat category, consumers are particularly sensitive to what is included in the ingredient list and often expect natural solutions to extend shelf life. Consumers are also making product choices based on the sustainability of ingredients, and this has contributed to the tremendous rise in plant-based meat alternatives. But what limits the shelf life of these products? Even though it looks and tastes like meat, does plant-based meat face the same oxidative stability challenges? To investigate, natural antioxidant strategies were tested in both meat and plant-based meat alternatives. In both cases, a widely used natural antioxidant (rosemary extract) was applied to the protein system, the samples were cooked, and then stored refrigerated. Analysis by gas chromatography (headspace aldehydes) showed a protective effect of rosemary extract in meat products over shelf life storage. A combination of natural antioxidants (rosemary and green tea), further increased stabilization of cooked meat products. These results were then positively correlated by a trained sensory panel identifying an increase in warmed-over flavor in the control meat treatments. When testing plant-based meat alternatives, headspace aldehydes did not increase over the storage of the samples, both with and without antioxidants. Results suggest that the production of other off flavors, possibly associated with proteins, are responsible for limiting shelf life. On the other hand, effect of microbial spoilage is very important in fresh meat-analog category on a separate trial and necessitates the usage of adequate food spoilage protection tools. Overall, the research emphasizes that understanding the matrix, and source of oxidizable lipids, is key in designing antioxidant strategies to extend product shelf life.

**Lipid Oxidation Challenges in Cell-based Seafood Manufacturing.** Lauran Madden, BlueNalu, USA; Rami Nasrallah, BlueNalu, USA

Cell-based seafood presents an opportunity to generate a new supply of seafood products with year-round availability and supply that supports growing global demand for healthy proteins. Improved consistency, shelf life, and quality can also be accomplished via the stabilization of lipid components. Inclusion of healthy fats during the process, such as EPA or DHA omega-3 fatty acids, is crucial to producing a product with a nutritional profile similar to conventional seafood products. Many studies report the negative impacts of lipid oxidation on sensory aspects of seafood products including odor, flavor, and texture. The rate of oxidation is dependent of the type of product as well as handling and storage conditions. In the manufacturing of cell-based seafood, cell growth and product formation are performed in a controlled environment with detailed specifications for raw materials and final product parameters. The use of antioxidants during key process steps can control oxidation processes both intrinsic to cells as well as from external factors. Cell growth in upstream manufacturing requires oxygenation of the environment and this process can result in the formation of reactive oxygen species (ROS). The control of ROS generation by antioxidants can improve cell growth as well as maintain quality of nutrients, such as lipids. In downstream manufacturing, antioxidants can be used to control chemical reactions in the product that may impact sensory characteristics. The selection of components must be carefully considered to provide the antioxidant function while retaining function of the cells and desired product sensory profile.

**Targeted Delivery of Omega-3 Long-chain Polyunsaturated Fatty Acids in Cultivated Seafood Products: Some Practical Considerations.** Brandon

Chen, Finless Foods, Inc., USA; Liyun Ye, Finless Foods, Inc., USA

In marine fish, omega-3 long-chain polyunsaturated fatty acids (omega-3) and their derivatives involve in many important cellular functions. The manufacturing of cell-based seafood bypasses the aquafarming step of the conventional seafood production; thus, during the cultivation phase the cells could be subjected to sub-physiological levels of omega-3 required by some marine fish. Therefore, supplementing omega-3 to the cells could support cell proliferation and tissue integrity. Omega-3 are desired nutritional components in conventional seafood with potential health benefits. Besides the nutritional value, omega-3 also serve as the precursors for the development of the characteristic fresh fish flavor through their interactions with lipoxygenases during harvesting and aging. Furthermore, omega-3 facilitate the partitioning and mass transport of desirable flavor molecules to the corresponding sensory receptors through their impact on the food matrices' physicochemical properties during mastication. In cultivated seafood products, the nutritional value and flavor characteristics imparted by omega-3 are expected to be the same as in conventional seafood. So, it is essential to incorporate and deliver omega-3 to an optimal distribution and composition mix where the appropriate physical, chemical, or biological reactions can take place.

Research on targeted delivery of pharmaceuticals and nutraceuticals in biological models has been ongoing for decades. For example, nanocarriers have been designed to deliver drugs. Similarly, food grade nanoemulsions are being utilized to control the gastrointestinal fate of bioactive compounds and the release of flavors. Existing research findings may guide us to develop delivery systems for omega-3 that preserve the maximum nutritional value, desirable fresh fish flavor, and essential biological functions for cells and tissues. However, challenges may come along the way, such as sourcing sustainable high-quality non-animal omega-3, oxidative stability of delivery systems, potential cytotoxicity introduced by nano-scale ingredients, and regulatory considerations to such ingredients in the final products.

**The Production of EPA and DHA in Transgenic Plants as a Sustainable, Environmentally Friendly Source of Omega-3 Fish Oils for Use in Novel Foods.** Johnathan Napier, Rothamsted Research, United Kingdom

There is wide-scale acceptance of the benefits of a diet containing oily fish as being preventative of metabolic pathologies such as CVD, and this health-protection is due to the presence of EPA and DHA, omega-3 long chain polyunsaturated fatty acids. These fatty acids are normally only present in the aquatic ecosystems, and as such, are sourced from either fish oils or microalgae. In the former, this requires environmentally undesirable depletion of ocenanic fish stocks, or in the latter, expensive culture by fermentation. A third alternative is to use transgenic plants engineered to make EPA and DHA as non-native enhancements to the seed oil profile.

Results: Transgenic Camelina sativa has been generated in which seven genes for the biosynthesis of EPA and DHA have been stably inserted into the plant genome. These genes (derived from microalgae) are under the control of seed-speciific promoters, ensuring that these non-endogenous fatty acids are only synthesise in seeds and during triacylglycerol deposition. Levels of up to 20% EPA+DHA has been routinely achieved in such plants and validated under "real world" conditions in multiple field trials (controlled environmental release of a GMO). Similarly, multiple aquafeed trials have demonstrated the safe and efficacious use of these novel feed as a dietary inclusion for important fish species such as salmon, tuna, trout etc.

Conclusion: The production of EPA and DHA in transgenic Camelina achieves levels of these important fatty acids similar or greater to that found in Northern Hemisphere fish oils, but without the concerns associated with co-accumulation of environmental pollutants or the impact on sustainability of marine fish stocks.

## Lipid Oxidation in Omega-3 Products

*Chairs Liyun Ye, Finless Foods, Inc., USA; David Johnson, Kalsec, USA*

**Chemical Compositional Changes in Over-oxidized Fish Oils.** Gerard Bannenberg, GOED Omega-3, USA; Austin Phung, University of California, USA; Claire Vigor, Université de Montpellier, France; Guillaume Reversat, Université de Montpellier, France; Camille Oger, Université de Montpellier, France; Martin Roumain, Louvain Drug Research Institute, Université catholique de Louvain, Belgium; Jean-Marie Galano, CNRS, France; Thierry Durand, Université de Montpellier, France; Giulio Muccioli, Louvain Drug Research Institute, Université catholique de Louvain, Belgium; Adam Ismail, KD Pharma / GOED Omega-3, USA; Selina Wang, UC Davis, USA

Administration during gestation of a highly rancid hoki liver oil, obtained by oxidation through sustained exposure to oxygen gas and incident light for 30 days, has been reported to cause newborn mortality in the rat (Albert et al. Am. J. Physiol. Regul. Integr. Comp. Physiol. 311; R497–504, 2016). These effects were attributed to lipid hydroperoxides formed in the omega-3 long chain polyunsaturated fatty acids-rich oil, while other chemical changes in the damaged oil were overlooked. In the present study the oxidation condition employed to damage the hoki liver oil was replicated, and the extreme rancidity achieved was confirmed. A comprehensive

analysis of temporal chemical changes resulting from the sustained oxidative challenge involved measures of eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA) omega-3 oil oxidative quality (peroxide value, para-anisidine value, and total oxidation number, acid value, oligomers, antioxidant content and induction time) as well as changes in fatty acid content, volatiles, isoprostanoids and oxysterols. The detailed chemical description was extended to refined anchovy oil, a more representative ingredient oil used in omega-3 finished products, as well as to a different type of oxidation involving thermal exposure in the dark in contact with air, an oxidation challenge which is more relevant to retail products. The two oils had different susceptibility to the oxidation conditions, resulting in distinct chemical oxidation signatures that were determined primarily by antioxidant protection as well as specific methodological aspects of the applied oxidative conditions. The concentrations of unique isoprostanoids and oxysterols markedly in the over-oxidized oils. These results help promote the understanding of the chemical changes associated with the deterioration of omega-3 EPA/DHA oils, and provide a broader base for future studies addressing the biological activity of oxidized edible oils.

**CROSS—A Clean Label Concept for Preventing Lipid Oxidation of Protein Isolates Recovered from Fish By-products.** Jingnan Zhang, Chalmers University of Technology, Sweden; Mehdi Abdollahi, Chalmers University of Technology, Sweden; Maire Alminger, Chalmers University of Technology, Sweden; Ingrid Undeland, Chalmers University of Technology, Sweden

pH-shift processing is a promising tool to recover functional proteins from fish by-products. However, lipid oxidation can be triggered due to the presence of PUFA and hemoglobin (Hb) combined with exposure to critical pH´s, affecting the quality of isolated proteins. Due to the wish for clean label and sustainable development within the food industry, we recently explored and showed good oxidation-inhibiting potential, of cross-processing herring and salmon by-products with other underutilized materials including lingonberry press cake, shrimp peels and brown seaweed (Saccharina latissima); all rich in antioxidants. The present study aimed at further studying the cross-processing concept by broadening the selection of antioxidant-rich supporting materials to include apple press cake, oat fiber residue, barley-spent grain and green seaweed (Ulva fenestrata). We also wished to investigate if cross-processing influenced the oxidative stability of produced protein isolates during extended storage on ice.

MDA, HHE and HNE levels in protein isolates stored on ice for up to 16 days revealed that all kinds of supporting materials, except shrimp peels, prevented lipid oxidation during cross-processing, although to different extents. Lingonberry press cake limited lipid oxidation to an extremely low level (6.83 $\pm$ 0.05 μmol MDA/kg) followed by apple press cake which reduced the maximum MDA content formed from 195.01 $\pm$ 11.44 μmol/kg to 137.88 $\pm$ 8.50 μmol/kg. The other materials only slightly delayed the production of aldehydes but failed to reduce the maximum level. The results indicated beneficial effects from cross-processing fish by-products with antioxidant-rich non-fish materials to minimize lipid oxidation both during cross-processing and during subsequent ice storage of protein isolates. The combination with lingonberry press cake was the most promising approach and will be subject for further studies.

**Development of Spray-dried Microcapsules as Efficient Omega-3 Delivery Systems for Food Fortification Purposes.** Nor E. Rahmani-Manglano, University of Granada (UGR), Spain; Irene González-Sánchez, University of Granada (UGR), Spain; Pedro J. García-Moreno, University of Granada (UGR), Spain; F. Javier Espejo-Carpio, University of Granada (UGR), Spain; Charlotte Jacobsen, Technical University of Denmark, Denmark; Emilia M. Guadix, University of Granada (UGR), Spain

The proven health benefits attributed to EPA and DHA have promoted the research and development of functional foods enriched with these bioactive compounds. However, due to the utterly low oxidative stability of omega-3 PUFAs, efficient delivery systems need to be designed for their successful incorporation to complex food matrices. In this study, the oxidative stability of fish oil-loaded microcapsules (ca. 13 wt% oil load) produced by spray-drying emulsions stabilized with whey protein hydrolysate (WPH) was investigated. WPH exhibits both emulsifying and antioxidant activity. The latter is highly beneficial to reduce lipid oxidation in heterogeneous systems. Glucose syrup (GS) or maltodextrin (MD21) were used as the encapsulating agents. The results showed that the GS-based microcapsules were the most oxidatively stable after 6 weeks of storage regardless of the storage temperature (4 °C or 25 °C), assessed based on peroxide value (PV) and secondary volatile oxidation products content (PV of 3.49 $\pm$ 0.25 meq O2/kg oil and a content of 1-penten-3-ol of 48.06 $\pm$ 9.57 ng/g oil after 6 weeks of storage at 4 °C). Moreover, the higher oxidative stability reported for the GS-based microcapsules containing WPH as film-forming material, compared to other studies, suggested a positive influence of the use of WPH as emulsifier with antioxidant activity. Additionally, low-fat mayonnaise enriched with: i) GS-based microcapsules containing WPH as film-forming material, ii) 5 wt% fish oil-in-water emulsion stabilized with WPH or iii) neat fish oil was produced. After the storage time (1 month, 25 °C), it was concluded that the delivery system played a major role on both the physical and oxidative

stability of the fortified matrix, being the mayonnaise enriched with GS-based microcapsules containing WPH as emulsifier the most oxidatively and physically stable.

**Impact of Microalgal Species on the Oxidative Stability of n-3 LC-PUFA Enriched Tomato Purees.** Robbe Demets, KU Leuven Kulak, Research Unit Food & Lipids, Belgium, Belgium; Lore Gheysen, KU Leuven Kulak, Research Unit Food & Lipids, Belgium, Belgium; Jolien Devaere, KU Leuven Technology Campus Ghent, Laboratory of Enzyme, Fermentation and Brewing Technology, Belgium, Belgium; Tom Bernaerts, KU Leuven, Laboratory of Food Technology, Belgium, Belgium; Ann Van Loey, KU Leuven, Laboratory of Food Technology, Belgium, USA; Luc De Cooman, KU Leuven Technology Campus Ghent, Laboratory of Enzyme, Fermentation and Brewing Technology, Belgium, Belgium; Imogen Foubert, KU Leuven Kulak, Research Unit Food & Lipids, Belgium, Belgium

Various studies have shown the potential of microalgae as a sustainable, alternative source of n-3 LC-PUFA. Incorporating them into the food chain by enriching food products could relieve pressure on the reducing fish stock and improve the daily intake of n-3 LC-PUFA in Western countries which is currently insufficient. For this, fruit and vegetable-based products are valuable matrices, since they are both part of a healthy diet and rich in antioxidants, which could be useful for overcoming stability issues regarding the lipid oxidation susceptibility of n-3 LC-PUFA. Also microalgae themselves contain diverse antioxidants that could be of importance. This study investigates the impact of microalgal species on the oxidative stability of tomato puree enriched with n-3 LC-PUFA from microalgae. For this, microalgae with clear differences in antioxidant profile and cell wall composition were selected: three photoautotrophs (Nannochloropsis, Phaeodactylum and Isochrysis) and one heterotroph (Schizochytrium). After enrichment of the tomato puree with the different microalgal biomasses (80 mg n-3 LC-PUFA/100 g puree), the enriched purees were characterized in terms of n-3 LC-PUFA, FFA, carotenoids, phenolic compounds, tocopherols and ascorbic acid, and were exposed to a 12-week storage experiment at 37°C. Both primary and secondary oxidation products were followed up to determine oxidative stability. Given the colored nature of microalgae, interference with standard analytical techniques for measuring PV was omitted by using an adapted FOX method. Volatile secondary oxidation products were determined using HS-SPME-GC-MS.

The results showed a significant influence of microalgal species: tomato purees enriched with photoautotrophic species were oxidatively stable whereas the one enriched with the heterotrophic one was not. Although rich in antioxidants, the tomato puree was therefore not able to keep all n3 LC-PUFA rich microalgae oxidatively stable. The endogenous antioxidants of photoautotrophic microalgae seemed to be of bigger importance to ensure oxidative stability.

**Lipid Oxidation in Innovative Protein Enriched Products from Cod- salmon and Herring Backbones During Ice and Frozen Storage.** Haizhou Wu, Chalmers University of Technology, Sweden; Mehdi Abdollahi, Chalmers University of Technology, Sweden; Ingrid Undeland, Chalmers University of Technology, Sweden

Several techniques, all applicable on fish co-products, are available today to separate muscle proteins from e.g., bones and skin, such as the pH-shift process, classic meat-bone separation, and enzymatic or non-enzymatic hydrolysis. However, applying such techniques on fish co-products is rendered difficult by their high susceptibility to lipid oxidation, and little is known today about which value adding process that fits best to which species of co-products. With this background, we studied the lipid oxidation development and its inhibition in protein enriched product from salmon, cod and herring backbones. Specifically, the influence of recovery technique (pH-shift processing vs mechanical separation), antioxidant addition and endogenous factors on lipid oxidation was investigated. Salmon-derived products were very stable during both ice and frozen (–20°C) storage, despite a small peroxide value (PV) development in pH-shift protein isolates (PI) at –20°C. Contrary, products from herring and cod backbones rapidly developed PV and TBA-reactive substances (TBARS) during ice storage, with the PI being most susceptible to oxidation in case of cod. At –20°C, herring PI developed less PV and TBARS than the corresponding mechanically separated meat (MSM), while for TBARS, the opposite applied to cod. In terms on general oxidation intensity, the three fish species studied followed the order: herring > cod > salmon, regardless of processing method. Pearson correlation tests showed a significant ($p < 0.05$) correlation between levels of Hb, total Fe, ascorbic acid, $\alpha$-tocopherol and lipid oxidation. Two antioxidant delivery strategies (pre-dipping backbones in Duralox MANC or its direct mixing into MSM and PI) remarkably increased the oxidation lag phase in both PI and MSM; from < 1 day to >15 days. The presented antioxidant strategies could hereby facilitate more diversified end-use of fish co-products from current fish meal to high-quality minces or PIs for the food industry.

**Physical and Oxidative Stability of High-fat Fish-oil Emulsions Added Algae-based Stabilizers from saccharina Latissima.** Ditte B. Hermund, DTU Food, National Food Institute, Denmark; Ioanna Anagnostara, DTU, USA; Xiaoru Hou, Danish Technological Institute, USA; Maria Dalgaard, Mikkelsen, DTU, USA; Nanna Rhein-Knudsen, DTU, USA; Anne S. Meyer, DTU, USA; Charlotte Jacobsen, Technical University of Denmark, Denmark

Enriching foods with long-chain n-3 polyunsaturated fatty acids (PUFAs) requires a delivery emulsion system, which is both thermodynamically and oxidatively stable.
Three types of polysaccharides from the brown alga Saccharina latissima and one commercial alginate (REF) were evaluated in this study as stabilizers; alginate (SA), fucoidan extract (SF, 10% purity), and laminarin extract (SL, 10% purity).
SA, SL and REF showed in vitro metal chelating ability in the order: SA (99%) >SL (78%) >REF (31%) (no data on SF). The difference in antioxidant activity of alginate was related to structural differences (M/G ratio).
A storage trial was conducted using 70% (w/w) fish oil-in-water delivery emulsions added NaCas (0.23wt%) as emulsifier with SA, SF, SL and REF (concentrations: 0.1–0.4wt%, C1-C4, SF only C2). A control with only NaCas were included. The physical (e.g., creaming and droplet-size distribution) and oxidative (peroxide value and volatiles) stability of the emulsions, were evaluated (12 days, dark at 20˚C).
Gravitational separation of emulsions was determined using creaming index (CI), and acceptable creaming had a CI< 5%. CI >5% was observed in some emulsions, typically at low stabilizer-concentration. Acceptable CI was found for; REF (all concentrations), SF=0.2wt% (C2), SL and SA at 0.3wt% (C3) and 0.4wt% (C4). No change in droplet size observed in emulsions during storage. Hence, creaming was due to flocculation.
In general, the oxidative stability decreased by the addition of REF, SA and SF (except for REF at C1), as prooxidant activity was observed. However, SA showed antioxidant activity against formation of 2-ethylfuran. Partial protein displacement at oil-water interface was discussed as possible reason for prooxidant activity of alginate, due to the location of alginate-chelated transition metals. SL showed no/low antioxidant activity in decreasing formation of volatiles. The antioxidant activity of SL was related to the presence of antioxidative co-extracted compounds such as peptides and polyphenolic compounds.

## Lipid Oxidation Variation with Specific Food Matrices

*Chairs Charlotte Jacobsen, Technical University of Denmark, Denmark; Cindy Tian, Kalsec Inc., USA*

**Are Phospholipid Content and Its Unsaturation Really Important in Lipid Oxidation of Muscle Food?** Haizhou Wu, Chalmers University of Technology, Sweden; Jianhao Zhang, Nanjing Agricultural University, China (People's Republic); Mark P Richards, University of Wisconsin-Madison, USA
Lipid oxidation is a major cause of quality deterioration in muscle food, causing undesirable changes in color, flavor and nutritive value. Numerous studies have shown that the amount of phospholipid and the elevated unsaturation of the acyl chains of phospholipids seem to play important roles regarding onset of lipid oxidation in muscle. However, in our previous study, the rate and extent of rancidity with reduced lipid content (≤0.1% phospholipids) was the same with those 6 times more membrane lipids in washed cod muscle in the presence of whole blood (Richards & Hultin, 2001). Moreover, hemoglobin oxidized washed tilapia lipids more effectively than washed cod lipids in spite of the fact that washed cod lipids were around 2.8 times more unsaturated (Richards et al, 2007). Thus, besides phospholipid content and its unsaturation, other factor(s) regarding lipid as substrates for oxidation could be relevant and vary among different muscle tissues (e.g., pork compared to fish muscle). In this study, the roles of lipid oxidation substrates and muscle microstructure in lipid oxidation were investigated in two muscle models (cod and pig). Added myoglobin (Mb) promoted lipid oxidation in washed cod muscle (WCM) but not in washed pig muscle (WPM). The differing microstructure of WCM e.g., more exposed fat cells or membrane of muscle cells compared to the ''denseness'' or ''wrapped'' structure of WPM, may have contributed to the better ability of Mb to facilitate lipid oxidation in the WCM. Added phospholipids with polyenoic indexes of 282 and 24 activated Mb as an oxidant similarly in WPM while added neutral lipids and added free fatty acids had little effect. It is suggested that muscle microstructure and accessibility of Mb to phospholipids play critical roles in relation to Mb-mediated lipid oxidation while the phospholipid content and its unsaturation were less impacting.

**Green Tea Extract Retards Lipid Oxidation in Margarine by Affecting Interfacial Organization.** Sandra Egger, Senna, Nahrungsmittel Gmbh, Austria; Sarah Fruehwirth, University of Vienna, Austria; Thomas Flecker, ThermoFisher, Austria; Miriam Ressler, FH, Joanneum, Austria; Dennis Kurzbach, University of Vienna, Austria; Franz Jirsa, University of Vienna, Austria; Jakob Windisch, University of Vienna, Austria; Nesrin Firat, SENNA Nahrungsmittel GmbH & Co KG, Austria; Marc Pignitter, University of Vienna, Austria
Margarine, a water-in-oil (W/O) emulsion with 80–90% fat, has gained popularity as low-cost alternative to butter in the food industry due to its high content of unsaturated fatty acids. While consumption of high amounts of unsaturated fatty acids are considered healthy, they are easily oxidized leading to rancidity, formation of off-flavors and reduced customer satisfaction. However, lipid oxidation in emulsion systems can be influenced by many factors, such as the fatty acid composition in the fat phase, the interface structure, the absence and presence of pro- and antioxidants in the fat and water phase and the storage and processing conditions.

Thus, it was aimed to investigate the impact of margarine-representative ingredients on the extent of lipid oxidation in four different types of industrial margarine stored at 15°C for 180 days. Besides the expected progress of lipid deterioration, whose extent depended on the lipid profile of the fat phase, acetone was shown, for the first time, to be generated at a concentration up to 791 ppb. Furthermore, additives, such as alpha-tocopherol, rosemary and green tea extract, affected lipid oxidation in the margarine samples. Green tea extract showed the most prominent antioxidant effect, even at lower concentration than commonly applied. While the combination of green tea extract with citric acid, beta-carotene or NaCl increased lipid oxidation compared to green tea extract alone, a combination of all ingredients showed a decrease of lipid oxidation to the same level as in the margarine fortified with green tea extract only. These results provide a glimpse of the possible complex mechanisms underlying lipid oxidation and its retardation in W/O-emulsions and the need for further studies to produce a more stable, healthier margarine and, at the same time, to decrease the costs associated with the lipid oxidation-induced food waste.

**Lipid and Protein Oxidation Monitoring in Pressurized Meat: Oxidation Pathways.** Claire Guyon, ONIRIS, France; Anne MEYNIER, INRAe, France; Marie de LAMBALLERIE, ONIRIS, France

The use of high-pressure processing is increasing in food industries, as a way to extend the food shelf life with slight modifications of their organoleptic properties. However, oxidation reactions have been might occur after pressurization of meat.

Beef meat is rich in iron, mainly derived from the myoglobin. As the pressure treatment may induce a modification of protein structure, after high pressure treatment different forms of iron could be involved in oxidation reactions.

Our objective was to assess various aspects of oxidation reaction: (i) fate of iron, (ii) protein oxidation, (iii) lipid oxidation) after different levels of pressure and during the subsequent refrigerated storage. Therefore, heme iron, dialyzable iron, Thiobarbituric acid reactive substances (TBARS), hexanal, carbonyled proteins and protein adducts were evaluated to highlight key targets and mechanisms. High pressure treatment and subsequent storage were achieved under low oxygen atmosphere.

Our results evidenced that the high-pressure treatment increased the release of free iron, which promotes in turn oxidation reaction (i) mostly on protein after high pressure, (ii) mainly on lipids during the subsequent refrigerated storage.

Further studies in presence of metal chelators can support this hypothesis.

Then in food matrices containing both proteins and lipids, oxidation of both components is important to assess to be able to control the extent of oxidation.

**Oxidative Changes in Shell-on-prawns (Pandalus Borealis) During Frozen Storage with and Without Artificial Light.** Hanne Jensen, Royal Greenland Seafood A/S, Denmark; Charlotte Jacobsen, Technical University of Denmark, Denmark; Niels Bøknæs, Royal Greenland Seafood A/S, Denmark; Lisbeth Hansen, Technical University Of Denmark, Denmark; Ole Mejlholm, Royal Greenland Seafood A/S, Denmark

Shell-on-prawns (SOP) are a delicacy product of the cold-water prawn Pandalus borealis. SOP are commonly stored at the retail in an illuminated display freezer for an extended period of time. Previous studies have shown that illuminated frozen storage causes rapid oxidation in SOP, which is expressed as a change in colour from a light, pinkish to a more pale, orange and finally yellow colour, together with odour and taste changes. Little is known about the underlying mechanisms of the changes occurring. The present study aimed to understand how and when the oxidation occurs in the presence and absence of light. A second aim was to identify relevant storage conditions and time for subsequent experiments with antioxidant addition to SOP. SOP (average oil content 3.32±0.32%) were packed in air and stored at –20.6±1.21°C with and without light for 84 days. Samples were taken every 14 days, followed by an investigation of oxidative stability (PV, secondary oxidation products, and α-tocopherol consumption).

Results showed an increase in PV from the initial 3.39±0.48 meq/kg O2/kg oil to 22.09±1.45 meq/kg O2/kg oil with light and 7.8±2.25 meq O2/kg oil without light. A difference between treatments was observed after 70 days of storage. No consumption of α-tocopherol was observed during the storage period. Colour measurements (CIELab) showed an increased L* value, and decreases of the a* and b* values. The colour changes could not be explained by pigment change, as the astaxanthin content was not affected by the storage. In line with the PV results, differences were observed in the concentrations of secondary oxidation products (2,3-pentadione, heptanal, 2-methyl-butanal, 3-methyl-butanal) between samples stored with and without light. It was noticeable that changes were not observed until after four weeks of storage.

The chosen storage conditions were sufficient to show the effect of illuminated storage on SOP products.

**Strategy for Managing Oils Oxidative Stability in Food.** Diliara Iassonova, Cargill Inc, USA

Food industry demands for oxidative and flavor stable oils resulted in development of several high oleic oils with superior to commodity oils performance. Rising consumer awareness promoted reformulation of synthetic antioxidants from a wide range of products with antioxidant mixes containing tocopherols, rosemary, green tea, grapeseed or other extracts.

This presentation is focused on several examples for managing oxidative stability in crackers, cookies,

snacks, frying and feed applications. Performance of specialty high stability oils were compared to commodity oils performance with and without antioxidants addition. Results are revealing benefits of product and application specific solutions. Advancement in packaging and supply chain management are opening new opportunities for effective oxidation control in food and feed.
Product developers consider a variety of approaches for managing oil oxidative stability in food applications ranging from selecting high stability oils, adding antioxidants, modifying packaging and processing steps. Each solution should be selected based on product and system needs and limitations.

**Using Natural Food Additives to Extend the Shelf Life of Snacks Containing Nuts and Seeds.** Henna Lu, Kalsec Inc., Europe Ltd, United Kingdom
Nuts and seeds appeared to be one of the most popular savory snacks in Western Europe recently. Many snacks such as nut bar, confectionary and baked snacks contains various type of nuts and seeds. Due to the high fat level, especially polyunsaturated fatty acids in some of the nuts and seeds (such as walnut, pecan, peanut, hazelnut, sunflower seeds, etc), they are susceptible to lipid oxidation and consequently affect their oxidative stability. Shelf life study of various nuts, seeds, nut trail mix and nut bars were conducted. Nuts and seeds were treated with and without natural antioxidants before roasting or making into nut bars (hazelnut bar or dates bars containing nut pieces). Subsequently, nuts, seeds, nut trail mixes and nut bars were subjected to accelerated shelf life (exposure to light, or storage at accelerated temperature). The oxidative stability was measured by Oxipres or monitoring secondary volatile oxidation products by GC-MS, etc. Stabilization strategies including the type of antioxidants (green tea, rosemary extract or tocopherol), addition method (spraying oil soluble antioxidant onto the seeds versus soaking the seeds in brine solution containing water dispersible antioxidant) was discussed. The obtained results showed that the stability of nuts and seeds varied depending on the storage condition, addition method, surface area of exposure, fat composition of nuts or recipes of nut bars. This study provides a valuable information to nut suppliers and consumer product companies to improve the stability of nuts and seeds containing products.

## Lipid Oxidation: Mechanisms and Consequences

*Chairs Karen Schaich, Rutgers University, USA; Morgan Kandrac, Rutgers University, USA*

**Carbonyl-amine Reactions as an Unavoidable Consequence of Lipid Oxidation in Foods.** Francisco J. Hidalgo, CSIC-Instituto de la Grasa, Spain; Rosario Zamora, CSIC-Instituto de la Grasa, Spain
Lipid oxidation initiates a complex cascade of reactions and produces many compounds that play a major role in food quality and safety. However, when this cascade of reactions occurs in foods, lipid oxidation consequences do not finish with the formation of the secondary reaction products. In fact, some of these secondary lipid oxidation products react with the nucleophiles present in the nearby. The result is the broadcasting of the oxidative damage to other food components. In addition to co-oxidation reactions in which either proteins or vitamins, among others, are involved, carbonyl-amine reactions are responsible for many desirable and undesirable consequences. Among them, this paper will discuss the role of lipid oxidation products on browning development, endogenous antioxidants formation, and flavors and off-flavors generation. These reactions will be discussed at a molecular level and compared with similar reactions produced by carbohydrate-derived reactive carbonyls. Obtained results show that once lipid oxidation has been initiated, many competitive reactions are produced with either detrimental or beneficial consequences for the food quality. In addition, only a detailed study that takes into account the reactions produced by lipids as well as the reactions produced by other food components will allow understanding the changes produced in the food.

**Epoxide Formation During Lipid Peroxidation.** Claus Schneider, Vanderbilt University, USA
4-Hydroxy-2-alkenals are a class of important toxic and signaling molecules formed during autoxidation of polyunsaturated fatty acids. The 15S-hydro(pero)xides of arachidonic acid give rise to 4-hydro(pero)xynonenal in lipid film autoxidation reactions. This reaction appears to be a major pathway to aldehydes but the mechanism(s) of chain cleavage during peroxidation of polyunsaturated fatty acids and their hydro(per)oxides have remained ill defined. During the course of analyzing thin-film autoxidation reactions of the 15S-hydroxide and 15S-hydroperoxide of arachidonic acid a series of epoxidized products was identified containing cis and trans epoxide moieties. The original 15S-hydro (pero)xide moieties were unchanged in the epoxide products. The epoxidation reactions were explained by peroxyl radical cross chain addition to a conjugated diene and subsequent cleavage of the peroxyl group by homolytic intramolecular substitution (SHi) yielding the epoxide moiety. Addition of the peroxyl radical to a diene moiety resulting in acyl chain dimerization and oligomerization competes with the propagation step of hydroperoxide formation by H-abstraction. Mechanisms involved in dimerization, epoxidation, and carbon chain cleavage (i.e., aldehyde formation) during lipid peroxidation will be discussed.

**Interplay Between Lipid and Protein Carbonyls During Oxidative Reactions.** Mario Estevez, University of Extremadura, Spain; David Morcuende, University of Extremadura, Spain; Teresa Antequera, University of Extremadura, Spain

Lipids and proteins are known to interact by a number of mechanisms during the onset of oxidative reactions. The understanding of the molecular basis of those mechanisms is essential to control the onset of these reactions and avoid their negative consequences in terms of food quality and safety. This concise review collects the most recent information on the co-oxidation of muscle lipids and proteins, the fundamental basis of their interactions and the consequences of scientific interest. Classic theories in food chemistry support that oxidation of muscle tissue is initiated in phospholipids owing to the high unsaturation degree of their fatty acids. However, current knowledge on redox biochemistry reveals that certain proteins and amino acids are more prone to be oxidized by the hydroxyl radical than polyunsaturated fatty acids. Hence, certain protein residues such as thiols are known to readily react with free radicals to protect other biologically relevant amino acids in proteins and simultaneously, protect lipids from biological membranes against oxidation. This antioxidant action of proteins is documented in meat and muscle foods, though under severe pro-oxidative conditions (heating, processing) lipid oxidation occurs concomitantly with thiol depletion and leads to the formation of species, such as malondialdehyde (MDA). At this stage, both lipid and protein radicals may be transferred and initiate oxidative reactions in neighbouring molecules of either chemical nature. MDA is known to bind through covalent linkages to amino groups in proteins (secondary protein carbonylation) and this molecular interaction has several relevant consequences including modification of protein functionality and impairment of protein digestibility. The reactivity of MDA with proteins at advanced stages of the reaction questions the value of this oxidation product as a reliable indicator of lipid oxidation. Furthermore, protein-bound MDA may affect the detection and quantification of primary protein carbonyls by using the classic dinitrophenylhydrazine method.

**Kinetic Modeling of Malondialdehyde Reactivity in Oil to Simulate Actual Malondialdehyde Formation upon Lipid Oxidation.** Angelique Vandemoortele, University Ghent, Belgium; Philipe Heynderickx, University Ghent, Belgium; Ludivine Leloup, University Ghent, Belgium; Bruno De Meulenaer, University Ghent, Belgium

Malondialdehyde has been widely used as a marker to measure lipid oxidation. Due to its high reactivity towards proteins and DNA, malondialdehyde is considered to be potentially toxic to humans. Besides, malondialdehyde is prone to aldol-type self-condensation and hydrolytic cleavage, leading to the formation of a dimer and oligomer, and acetaldehyde and formic acid, respectively. In our previous study, surprisingly a high reactivity of malondialdehyde was observed in saturated oil. About 1.5 and 68% of the added malondialdehyde reacted by aldol self-condensation after 24h at respectively 4 and 40 °C. However, no temperature effect was observed for the hydrolytic cleavage of malondialdehyde in oil which accounted for 7% loss. This was quite contradictory with the multitude of reports found in literature on the increasing malondialdehyde levels in foods upon progressive oxidation. Therefore, the malondialdehyde reactivity in oil was further studied in order to describe the kinetics of its reaction pathways over a wide temperature range (25–180°C), typically used during food preparation and storage. The kinetic parameters were estimated using a global one-step non-linear regression approach. Finally, the actual MDA formation during thermal lipid oxidation of commercial sunflower oil was simulated. With respect to the non-ideal character of a lipid medium, a kinetic model was proposed that described the experimental malondialdehyde data by a reversible hydrolytic cleavage and an irreversible aldol self-condensation reaction. The latter reaction showed to be the main degradation route of malondialdehyde in oils. Simulation of the malondialdehyde formation during lipid oxidation of sunflower oil demonstrated that, depending on the heating time, the experimental malondialdehyde concentrations can substantially underestimate the ongoing lipid oxidation (e.g., after 8h at 120°C >80% of the formed malondialdehyde was not detected). In conclusion, the measured MDA level in oils should be considered with care, especially when high temperatures are applied (e.g., frying).

**Lipid Oxidation: Where Have All the Products Gone? A Wholistic Look at Lipid Oxidation.** Karen Schaich, Rutgers University, USA

Lipid oxidation is far more complex than the simple free radical chain reaction normally depicted, yet we persist in following lipid oxidation by limited products. When monitoring only hydroperoxides with perhaps added volatile hexanal, we have observed many cases where lipid oxidation appears to be negligible or low in measured products while color, odors, and flavors suggest active oxidation. Where are the products? The obvious explanation for this anomaly – that active oxidation pathways and their products were just missed – is an oversimplification that assumes all lipid oxidation products are generated then just accumulate in the system waiting to be detected. However, lipid oxidation products are in constant flux. They decompose and transform to compounds that may not be expected so are not detected in targeted assays or are ignored in broader assays such as GC-MS. One example here is aldol condensation of aldehydes that converts aldehydes to polymers. Just as importantly, nearly all lipid oxidation products co-oxidize other molecules, particularly antioxidants and proteins, generating still different lipid products that just get classified as ‘‘unidentified’’ while removing epoxides, aldehydes and other lipid products from the analytical stream as adducts are

formed. These footprints of lipid oxidation are not detected in standard oxidation analyses but contribute significantly to browning, release of off-flavors and odors, texture modifications, loss of nutrient value, formation of acrylamide and other toxic products, and more. As it provides a wholistic overview of lipid oxidation and stretches past hydrogen abstractions and hydroperoxides to broadcast oxidation throughout foods, this paper will set the stage for all LOQ papers of this meeting stretching our thinking and looking beyond the obvious.

## Lipid Oxidation in Interesterified Fats and Oils and New Oil Products

*Chairs Ignacio Vieitez Osorio, PEDECIBA Quimica-UdelaR, Uruguay; Dilip Nakhasi, Stratas Foods LLC, USA*

**Caffeoyl Fatty Acyl Structural Esters: Enzymatic Synthesis, Characterization and Antioxidant Assessment.** Longmei Weng, South China University of Technology, China (People's Republic); Lin Li, South China University of Technology, China (People's Republic); Lili Ji, South China University of Technology, China (People's Republic); Zuowei Xiao, South China University of Technology, China (People's Republic); pengyao sun, South China University of Technology, China (People's Republic); Zhiyi Chen, Sericultural & Agri-Food Research Institute, China (People's Republic); Di Zhao, South China University of Technology, China (People's Republic); Bing Li, South China University of Technology, China (People's Republic); Xia zhang, South China University of Technology, China (People's Republic)

A novel two-step method of lipase-catalyzed synthesis of caffeoyl fatty acyl structural esters was developed. The hydrophilic 1-monocafferol glycerol(1-MCG) and hydrophobic monocaffeoyl monofatty acyl glycerols (MCMFGs) were synthesized, purified, and identified. The antioxidant properties of 1-MCG & MCMFGs increased by the improved dispersity and decreased by the ''block'' effect of their fatty acyl groups on catechol group

Objective: The objective of this study is to synthesize 1-MCG and MCMFGs with promising dispersity and antioxidant properties.

Methods: The 1-MCG was firstly synthesized by Lypozyme 435 catalysis between ethyl caffeinate and glycerol. Afterwards, the 1-MCG further reacted with fatty acids to generate MCMFGs. Monocaffeoyl monopalmitoyl glycerol (MCMPG), monocaffeoyl monooleoyl glycerol (MCMOG) and monocaffeoyl monolinoleoyl glycerol (MCMLG) were respectively synthesized with different fatty acids. The transesterification, esterification and hydrolysis processes were monitored by HPLC-UV. Then, 1-MCG and MCMFGs were purified by column and preparative chromatography, respectively, and their structures were verified by HPLC-MS/MS and NMR. Eventually, the DPPH and ABTS assays were applied to estimate their antioxidant properties.

Results: High ethyl caffeinate/1-MCG conversion ( >98.26%) and the maximum yields of 1-MCG (80.99%), MCMPG (26.26%), MCMOP (35.37%) and MCMLP (45.43%) were obtained. The HPLC-MS/MS and NMR results suggested successful synthesis of 1-MCG and MCMFGs ( >95%). The ionization and viscosity were important during the synthesis process. Subsequently, the antioxidant assays supported the 1-MCG and MCMFGs as effective antioxidants and the ''block'' effect of fatty acyl groups on the catechol group in MCMFGs might result in the varying scavenging properties.

Significance: This study lighted not only the application of 1-MCG & MCMFGs in industry, but also the further design of phenol lipids.

**Chia Seed Oil: Cold Pressing Extraction, Oxidative Stability, Vehiculization and Its Application in Food.** Marcela Lilian Martinez, CONICET, Argentina; Agustín Gonzalez, CONICET, Argentina; María Gabriela Bordón, CONICET, Argentina; María Cecilia Penci, CONICET, Argentina; Romina Mariana Bodoira, CONICET, Argentina; Pablo Daniel Ribotta, CONICET, Argentina

Chia seed is source of linolenic and linoleic fatty acids, 54–67% and 12–21 %, respectively. A diet rich in polyunsaturated fatty acids decreases the risk of many chronic non-communicable diseases. The incorporation of omega-3-rich oils to other vegetable oils and processed foods seems to be an efficient way to increase its consumption, however, when these are exposed to enviromental and process conditions, oxidation reactions occur. Many extraction techniques have been employed to remove chia oil preserving its oxidative stability. The 82.2 % of the available oil was recovered by screw pressing showing very low values in free fatty acid and hydroperoxides. Several strategies as the addition of antioxidants, oil blending and microencapsulation procedures were studied in order to enhance lipids' stability. Blending chia with other oils represents a way to produce new distinctive oil products. In our experience, blends with sesame oils were the most stable due to a synergistic effect between total tocopherols and lignans present in the oil. Finally, the microencapsulation of chia oil generates a dual benefit because it provides not only a protective effect of the active compounds, but also produces a change in the physical state from liquid oil to a flowing powder. Given the importance of the consumption of bakery and pasta products, the incorporation of microencapsulated chia oil in these food groups were investigated. Our studies demonstrated that the inclusion of microencapsulated

chia oil does not modify the technological quality and cooking parameters of bread and durum wheat dry pasta. In addition, microencapsulation exerts a protective effect on the oil against the oxidation leading to a safer product. After these studies we could affirm that chia oil blending and microencapsulation allow the development of omega-3 enriched systems, new oils and food products such as bread and dry pasta, respectively.

**Improving Oxidative Stability of a Menhaden-oil Based Butterfat Analog Using Phenolic Compounds as Antioxidants.** Siyu Zhang, University of Georgia, USA; Sarah Willett, Kerry, USA; Joseph Hyatt, University of Georgia, USA; Silvana Martini, Utah State University, USA; Casimir Akoh, University of Georgia, USA

Phenolic compounds, including gallic acid, propyl gallate, 1-o-galloylglycerol (GG), ferulic acid, caffeic acid, rosmarinic acid, carnosic acid, tocopherol mixture (TOC), and butylated hydroxytoluene (BHT), were investigated as antioxidants to improve the oxidative stability of a structured lipid (SL) produced through the enzymatic acidolysis of menhaden oil with caprylic and stearic acids. Lipozyme® 435, a commercially available food grade lipase was the catalyst. BHT was chosen as the positive control. The SL was rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and had similar melting point, solid fat content, thermal behavior, rheological property, and polymorphism as butterfat. However, due to its high polyunsaturated fatty acid (PUFA) content, the SL was more susceptible to oxidation than butterfat. To increase its oxidative stability, the above phenolic compounds were added to SL as antioxidants at 100 ppm, respectively. The samples were evaluated for peroxide, p-anisidine, total oxidation (TOTOX) values, change in fatty acid composition, and hydrolysis of triacylglycerol during an 18-day accelerated oxidation test at 70 °C. GG, RA, and BHT showed significantly higher improvement regarding the oxidative stability of SL than the rest of the compounds with the TOTOX values around 250 after 18 days. Oxidation induction time (OIT) using differential scanning calorimetry (DSC) also showed a strong correlation with the antioxidant effects of the phenolic compounds in the accelerated oxidation test. A mixture of GG and TOC at 50:50 ppm gave the best antioxidant effect on SL (OIT = 115.1 min) compared to the other combinations at the same concentration, including rosemary extract at 500 ppm (OIT < 100 min).

**Novel Lecithin-based Oleogels and Oleogel Emulsions Delay Lipid Oxidation and Extend Probiotic Bacteria Survival.** Xiaoqing Zhuang, Iowa State University, USA; Nicole Gaudino, Iowa State University, USA; Stephanie Clark, Iowa State University, USA; Nuria Acevedo, Iowa State University, USA

The oxidation of edible oil produces both primary and secondary oxidation products including hydroperoxides, carbonyls, hydrocarbons, and epoxides, which negatively affect their sensory and biological properties. As a result, inhibition of lipid oxidation in foods is of great importance. It has been shown that phospholipids can enhance the viability of probiotics by binding to them; however, undesirable off-flavors of oxidized phospholipids might hinder their use in food industries. The objective of this study was to formulate a semisolid soy lecithin oleogel emulsion to entrap probiotics while preventing the progress of lipid oxidation. Oleogel (OG) and oleogel emulsions (OGE) were formulated with different ratios of soy lecithin (SL) and stearic acid (SA) with 1 wt% or 10 wt% water. The oxidation level of OG and OGE was determined by measuring peroxide value (PV) over 60-day storage time at 20°C. Compared with an unstructured system, OG and OGE successfully delayed the progress of lipid oxidation. Furthermore, OG and OGE containing SL did not oxidize throughout storage time. The delay of the lipid oxidation progress in OG and OGE can be attributed to the physical entrapment of oil via the lecithin fibrillar network, which immobilized the oil and thus prevented the contact with prooxidant molecules, and the soy lecithin in OGE enhanced probiotic survival as well.

**Oxidation Status of Encapsulated Structured Lipids Applied in Kefir Product.** Alev Yüksel-Bilsel, Istanbul Galata University, Turkey; Neşe Şahin-Yeşilçubuk, Istanbul Technical University, Turkey

Introduction: Although there is increasing demand for enrichment of foods with long-chain polyunsaturated fatty acids (LC-PUFAs) containing oils, avoiding lipid oxidation still exist as a major challenge for food producers. Therefore, oil encapsulation technology has been increasingly attracting the attention to retard lipid auto-oxidation and increase the range of applications.

The objective of this study was to investigate the oxidative stability of encapsulated MLM-type structured lipids (SLs) and to assess the oxidative stability when incorporated into kefir product.

Method: MLM-type SLs containing FAs of Echium oil at sn-2 position and caprylic acid at sn-1,3 positions were encapsulated by complex coacervation method in the presence of gelatin and gum Arabic. Effect of transglutaminase enzyme was also investigated as cross-linking agent. Oxidative stability tests (peroxide value, p-anisidine value) were conducted for the freeze-dried coacervates and coacervate suspensions of SLs during 30 days of storage at 4 °C and ambient temperature. Kefir samples fortified with complex coacervation products were analysed for their stability during storage for 10 days at 4 °C. Moreover, in vitro release profile of encapsulated SL in kefir samples were conducted.

Significance: In our study, MLM-type SLs were added into kefir product and oxidative stability tests were

conducted for the first time. In addition, release study was applied to encapsulated MLM-type SL for the first time to determine the possible food matrix effect.
Results: The kefir sample fortified with free SL had the lowest oxidation value whereas coacervate and suspension fortification of kefir resulted in higher oxidation levels throughout the 10 days of cold storage. Moreover, transglutaminase-treated coacervates and suspensions were more vulnerable to lipid oxidation than non-treated coacervates and suspensions. Based on the findings, kefir was found to have no significant matrix effect on oil release kinetics of encapsulated SLs ($p > 0.05$).

**Synthesis of Structured Lipid by Fast Acidolysis Catalysed by yarrowia Lipolytica Lipase in Solvent-free Medium.** Alexandre Torres, Lipidomics and Lipid Biochemistry Laboratory, Brazil; Emilia Akil, Federal University of Rio de Janeiro, Brazil; Adejanildo Pereira, Federal University of Rio de Janeiro, Brazil; Tatiana El-Bacha, Federal University of Rio de Janeiro, Brazil; Priscilla Amaral, Federal University of Rio de Janeiro, Brazil
The structured lipid consisting of medium-chain fatty acids and oleic (18:1n-9) acid in positions sn-1,3 and sn-2 of the glycerol backbone, respectively, was produced by acidolysis in solvent-free medium. Yarrowia lipolytica lipase, free and microencapsulated, was used as the biocatalyst, and the substrates were the free fatty acids (FFA) capric (10:0) and lauric (12:0), and triolein or olive oil as sources of triacylglycerols (TAG). Medium-chain fatty acids were rapidly incorporated into the sn-1,3 positions with both TAG substrates, up until 30 % of incorporation efficiency (IE%) of capric and lauric acids into structured lipids. Y. lipolytica lipase showed a strictly sn-1,3-regioselective profile in acidolysis reaction, confirmed by nuclear magnetic resonance and chromatography following pancreatic lipase hydrolysis. The FFA:TAG ratio affected the IE% and it was FFA-specific, as doubling the ratio from 2:1 to 4:1 increased IE% to 90% of the 12C FFA, exclusively. Immobilization of the lipase by microencapsulation in chitosan-alginate beads resulted in similar incorporation efficiency for lauric acid into olive oil TAG and this reaction could be performed for five cycles without losing its catalytic activity. Y. lipolytica lipase showed promising features for future use in the production of bioactive structured lipids and its use might benefit from the recent technological interest in the enzymatic synthesis of structured lipids, represented by the increasing patents' priority and publication, especially in Asia.

## Lipid Oxidation and Quality Poster Session

**Antioxidant activities and chemical profiles of aqueous and ethanolic extracts from heated sesame meals in oil-in-water emulsion.** HeeBin Seo, Sungkyunkwan University, Republic of Korea; SoYoon Park, Sungkyunkwan University, Republic of Korea; KeunCheol Yoo, Sungkyunkwan University, Republic of Korea; JiHee Hong, Sungkyunkwan University, Republic of Korea; Seung-Ok Yang, Seoul National University, Republic of Korea; JaeHwan Lee, Sungkyunkwan University, Republic of Korea
Sesame meal is a byproduct of sesame oil processing and contains high percentage of protein, lignans and sesamol. Therefore, many researches have been conducted to utilize sesame meal to get value-added biomaterials. However, studies using oil-in-water (O/W) emulsions as matrix are limited in the literature, although O/W emulsion is one of major food matrices with unsaturated fatty acids.
The objectives of this study were to determine chemical profiles of heated sesame meal and to evaluate antioxidant properties of extracts from heated sesame meal in O/W emulsion.
Comprehensive chemical profiles of extracts were conducted using GC/MS after derivatization. Sesame meal was heated at 0, 140, and 170°C for 1 h and the aqueous and 70% ethanol extracts were obtained. O/W emulsion containing 100 ppm extracts were stored at 50 °C for 2 weeks and degree of oxidation were evaluated.
β-Gentiobiose and sucrose were two dominant chemicals in aqueous and ethanolic extracts. Generally, heat treatment decreased the contents of amino acids, organic acids, carbohydrates and its derivatives. As heating temperature increased, sesamol concentration increased while sesamolin and sesamin decreased in both sesame meal extracts. As temperature of heat treatment increased, oxidative stability of O/W emulsions containing both extracts increased at 50 °C storage.
Sesame meal extracts received thermal treatment possessed higher antioxidant properties in O/W emulsion partly due to the generation of sesamol. Therefore, sesame meal extracts are suitable in enhancing self-life of O/W emulsion based food products as natural ingredients.

**Chemical characterization of Echium plantagineum seed oil obtained by three methods of extraction.** Giovanna Carlini, 1LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Inar Castro, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Gabriela Roschel, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Roseli Ferrari, Institute of Food Technology (ITAL), Campinas, SP, Brazil, Brazil; Severino Alencar, Department of Agri-Food Industry, Food & Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Brazil; Helton Ota, LADAF, Department of

Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Brazil; Tayse Ferreira, LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, USA

Echium seeds oil has been considered an important alternative to marine oils as source of omega 3 fatty acids, due to the high proportion of stearidonic acid (C18:4 n3). The process applied on the seeds can affect chemical, physical, functional and sensory characteristics of the extracted oil. Thus, our aim was to determine the chemical and sensory parameters of the oil obtained from Echium plantagenium seeds submitted to three extraction techniques: continuous screw press (PRESS), hydraulic press (HYD), and hexane extraction. In addition, a mixture (BLEND) was prepared containing 70% PRESS oil and 30% of the oil extracted with hexane. Partial results showed that peroxide value (mmol Kg-1 oil) increased from PRESS ($0.61 \pm 0.01$) and HYD ($0.86 \pm 0.01$) to BLEND ($1.69 \pm 0.01$) samples, but were below the Codex Standard limit for unrefined oils (7.5 mmol Kg-1 oil). HYD oil presented the highest concentration of malondialdehyde ($351.97 \pm 8.40$ μmol Kg-1 oil) compared to PRESS ($173.13 \pm 12.93$ μmol Kg-1 oil) and HYD ($173.76 \pm 4.70$ μmol Kg-1 oil) oils. Gamma-tocopherol was found in the higher amount in the PRESS oil ($779.21 \pm 1.46$ mg/Kg oil). In terms of phenolic compounds, rosmarinic, gallic and caffeic acids, as aglycones or glycosides derivatives were found in a higher frequency in the samples, according to the qualitative LC-ESI-QTOF-MS analysis. Although chemical differences have been observed, sensory characteristics were similar among the oils, that presented a general acceptability score of $5.95 \pm 0.14$ in a scale from 1 to 9, being the green color detected as a negative aspect. Plant sterols and volatile compounds profile will be determined in the next step by GC/MS, providing a better chemical characterization and oxidative stability approach of this important vegetable alternative to fish oil consumption.

**Comparative assessment of native and micellized forms of antioxidants' influence on the shelf life of the emulsion fat product.** Anatoly Samoylov, Aquanova RUS, Russia; Julia Nikolaeva, MSUFP, Russia; Alexey Nechaev, MSUFP, Russia

Based on a social plight for the use of natural antioxidants in the food industry, tocopherols, their various mixtures, as well as the use of ascorbic acid to prevent oxidation are widespread. As most natural antioxidants have lower antioxidant activity in fats than synthetic ones and, in most cases, require more complex incorporation into the product, their widespread industrial application can negatively affect organoleptic properties and the cost of the finished emulsion fat product. One way to solve this problem is to use new micellized forms of antioxidants. The micellization of various types of water- and fat-soluble antioxidants can increase their specific activity, which is apparently associated with an increase in the relative interaction surface in the product.

Comparative assessment of the effectiveness of natural antioxidants in native and micellized forms in the margarine formulation.

Antioxidants (tocopherols, rosemary extract, and ascorbic acid) in the native and micellized forms were selected as objects of study for their introduction into margarine. During storage of margarine in its fatty phase were determined PV and p-AV in dynamics.

Experimental data have been obtained on the action of the studied antioxidants of various technological forms on the formation of primary and secondary oxidation products in margarine during storage. It was determined that the introduction of micellized forms of various antioxidants (ExtraOx®MTA0117Advanced and ExtraOx®RATA0109) into margarine at a concentration of 0.015–0.03% most effectively slows down the formation of oxidation products compared to using their native forms in an amount equivalent to the active substances.

It has been experimentally established that the use of micellized forms of tocopherols, ascorbic acid and rosemary extract allows more efficient creation of emulsion fat products with an extended shelf life.

The practical significance of this study is the possibility of using micellized forms of antioxidants in various fat and oil products.

**Comparative effect of antifoaming agent polydimethylsiloxane and synthetic antioxidant tertiary butyl hydroquinone on thermal oil degradation in deep frying.** Mayamol Nichlavose, Emirates Refining Co. Ltd, IFFCO, United Arab Emirates; Rupesh Sarfare, International Food Stuffs Company (IFFCO), United Arab Emirates; Sergey Melnikov, IFFCO, United Arab Emirates

Frying oils used continuously or repeatedly at high temperatures are subject to a series of multiple chemical reactions, e.g., thermal oxidation, hydrolysis and polymerization, which lead to the formation of a variety of oil decomposition products. These substances may have adverse effects on the sensory and/or nutritional quality of fried foods and may also be harmful to human health. Natural and synthetic antioxidants (AOX) are commonly added to frying oils to reduce their oxidation during shelf life and upon their use at high temperatures. Although consumers have preference for natural AOX due to their superior authentic perception, in-use cost and antioxidative efficiency considerations often result in the use of synthetic AOX in industrial frying oils. At the same time, there is a clear trend to minimize the content of synthetic AOX in frying oils while maintaining their shelf life and in-use performance.

The objective of the present study was to quantify the effect of an antifoaming agent polydimethylsiloxane, PDMS, in the presence and in the absence of a synthetic antioxidant tert-butylhydroquinone, TBHQ, on the frying stability of refined sunflower oil. AOCS official methods were used throughout the study for the assessment of frying oil quality: Oil decomposition products were monitored by following key markers of oil deterioration, e.g., total polar compounds (TPC) and free fatty acids (FFA) content, p-anisidine value (AV) and oil color intensity. Our study shows that PDMS is highly efficient for the control of all oil thermal degradation markers upon repeated/continuous frying both in the presence and in the absence of TBHQ. The reasons of additive or synergetic effects of PDMS and TBHQ on individual markers are discussed. The outcome of our investigation allows to design frying oils with an optimal balance between shelf life stability and in-use oxidative stability while minimizing the use of synthetic AOX.

**Determination of shelf-lives of various virgin oils by the OXITEST method as a substitution for the Schaal oven test.** Chieh-Hsi Tsao, Taipei Medical University, Taiwan (Republic of China); Wei-Ju Lee, Taipei Medical University, Taiwan (Republic of China)

When analyzing the shelf-life of edible oil, the Schaal oven test (AOCS Cg 5–97) is commonly recommended to obtain shelf-life by determination of induction periods (IPs) in three measurements at three relatively low temperatures (60–80°C) through monitoring peroxide value and made an exponential oxidation graph. However, since it is not an automated test, the Schaal method is variable in the case of repeated handling and not ideal as a routine analysis system. The aim of this study is to examine a newly published method, the OXITEST method (AOCS Cd 12c-16), for determining the shelf-life of edible oil to compare with the Schaal method. The parameters for the OXITEST method were optimized as follows: sample amount of 5 g oil, oxygen pressure at 8 bar, and a linear regression curve with temperatures ranged from 60 to 100°C. The IP acquired by the OXITEST method (IP1) was shorter than all of those obtained by the Schaal method via peroxide index (IP2), p-anisidine index (IP3), and sensory evaluation (IP4), in an order of IP1< IP2< IP3< IP4. The bias closely matched the lipid oxidation mechanism, making OXITEST an attractive alternative due to the most conservative estimation it made prior to oil oxidation. Shelf-lives at 25°C of fourteen expeller-pressed seed oils were estimated by the OXITEST method, showing a very wide range between 85 days for brown linseed oil and 1089 days for peanut oil. In addition, as expected, the length of shelf-life of virgin oil attributed to multiple factors could not be simply predicted by either physical or chemical property (i.e., acid value, smoke point, and iodine value). These findings suggest that the OXITEST method is practical and promising for the measurement of the shelf-life of edible oil to ensure its quality and safety.

**Distribution of aldehydes compared to other oxidation parameters in oil matrices during autoxidation.** KeunCheol Yoo, Sungkyunkwan University, Republic of Korea; JaeHwan Lee, Sungkyunkwan University, Republic of Korea; ChanHyung Kim, Sungkyunkwan University, Republic of Korea; HeeBin Seo, Sungkyunkwan University, Republic of Korea; SoYoon Park, Sungkyunkwan University, Republic of Korea; JiHee Hong, Sungkyunkwan University, Republic of Korea

Aldehydes are typical secondary oxidation products generated from β-scission of lipid hydroperoxides (LOOHs). Aldehyde contents can be used to determine the degree of lipid oxidation of oil rich systems. Recently, prooxidative roles of aldehydes are reported. To understand roles of aldehydes in lipid oxidation, difference in aldehyde distribution between inner matrix and headspace should be understood first. Correlations among aldehydes and other typical oxidation markers in oil matrices during autoxidation also need to be evaluated.

The objectives of this study were to evaluate the distribution of aldehydes including propanal, hexanal and nonanal between IM and HS in bulk oils and O/W emulsion and to compare changes of aldehyde contents with typical oxidation parameters in soybean oil and O/W emulsion at 50°C of autoxidation condition.

Distribution of aldehydes and other oxidation parameters among headspace and inner matrix were determined in soybean oil or O/W emulsion during 50°C autoxidation.

Bulk oil matrix had higher portion of IM aldehydes than O/W emulsion. HS aldehydes in O/W emulsion reflected aldehyde content better than in bulk oil. Moisture content in soybean oil increased distinctively before the generation of oxidation products including hydroperoxides and volatiles. HS aldehydes and other oxidation parameters were simultaneously increased in soybean oil. In case of O/W emulsion, contents of HS aldehydes, not lipid hydroperoxide, increased linearly during autoxidation.

HS aldehydes reflected oxidation stage better in O/W emulsion than in bulk oil based on partition distribution and linear changes during autoxidation. Role of aldehydes should be carefully evaluated in bulk oils or oil-rich foods.

**Efecto de los ciclos de fritura a presiones reducidas sobre la calidad del aceite de salvado de arroz.** Nadia Segura Herrera, UDELAR, Uruguay; Ana Borche, UDELAR, Uruguay; Bruno Irigaray, UDELAR, Uruguay; Ivan Jachmanian, UDELAR, Uruguay

Frying at reduced pressures is a well-known technology that allows producing foods with similar characteristics

to traditional frying, but with lower lipid content. Additionally, the lower temperature required compared to the conventional process, extends the life of the frying oils, which also depends on oil origin, composition and antioxidants content.

In the present work, the behavior of rice bran oil exposed to a series of thermoxidation cycles simulating the loading and unloading of a vacuum fryer equipment was studied.

Each cycle comprised the following steps: pressure reduction to 90torr, heating under reduced pressure to 130°C, a 30min isothermal period, 30min period at 130°C and room pressure, cooling to room temperature. Oil samples were taken every 8 cycles and the content of polar compounds (PC), polymers, and natural antioxidants (tocoles and oryzanols) and the peroxide value (PV) were determined. Thermoxidation process was continued until the content of PC exceeded 25%.

A continuous increase in PC and polymers´content was observed, reaching values of 21% and 4% respectively at the end of cycle#32, values above the limits recommended in most of the food regulation agencies for both parameters in frying oils.

Regarding natural antioxidants, it was found that already in cycle#8 the content of tocopherols, tocotrienols and oryzanols decreased to 33%, 50% and 50 % of their initial concentration, respectively.

The PV progressively increased reaching a maximum of 35 meqO2/Kg after cycle#16, after which started to decrease.

Results suggest that, although it is expected that under reduced pressure, the deterioration of the oil is slowed down, the periods at room pressure promoted primary oxidation and the degradation of the antioxidants. Thus, even oil meets the limits concerning the content of polar and polymeric compound, it can exceed the limits of PV recommended for edible oils and should not be suitable for consumption.

**Effect of feeding olive pomace acid oil on lipid composition and oxidative stability of chicken and pork meat.** Paula Albendea, Universitat De Barcelona, Spain; Alba Tres, Universitat de Barcelona, Spain; Gerard Verge-Merida, Universitat Autonoma de Barcelona, USA; Ana Cristina Barroeta, Universitat Autonoma de Barcelona, Spain; Marçal Verdú, BonÀrea Agrupa, Guissona, Spain; Stefania Vichi, Universitat de Barcelona, Spain; Magdalena Rafecas, Universitat de Barcelona, Spain; Francesc Guardiola, Universitat de Barcelona, Spain

Acid oils (AO) are fat by-products from edible oil refining, characterized by their high proportion of free fatty acids (FA). For years, there has been an interest in using them in animal feeding because of their high energetic value.

The aim of this study was to evaluate if the inclusion of olive pomace AO in feeds had any significant effect on FA composition, tocopherol and tocotrienol (T and T3) profile and TBA values in chicken and pork meat.

Broilers (Ross 308) and pigs [(Landrace x Large white) x Duroc] were fed the growing-finishing period (18 and 61 days, respectively) 3 dietary treatments including 6% of palm oil (P), olive pomace oil (OP) or olive pomace acid oil (OPA). After slaughtering, samples of meat with skin from deboned legs of broilers and samples of longissimus dorsi muscle of pigs were taken (8 analytical replicates per treatment in both cases).

FA composition, tocol (T and T3) profile and TBA value were determined in fresh and refrigerated meats under commercial conditions: O2/CO2; 70/30; 3–4°C; 7 days for poultry and 8 days for pork.

FA composition of pork and chicken meat was influenced by the dietary treatment. Tocol profile and total tocol level depended on the type of meat and the dietary treatment. Total tocol concentration was higher in chicken than in pork. In chicken, the highest tocol level was found in OPA and P samples, while in pork the highest was in P and the lowest in OPA meats. Refrigeration storage decreased total tocol levels in pork but not in chicken. Dietary treatment and type of meat influenced TBA values, which showed higher levels after the refrigeration.

Studying the use of AO in animal feeding can contribute to the sustainability of the food chain.

**Effect of various tocopherol sources on the stabilization of animal fats.** Florian Henne, Kemin Nutrisurance, Italy; Liesbet Van Gils, Kemin Nutrisurance, Belgium; Vera Biermans, Kemin Nutrisurance, Belgium; Cristina Murcia Garcia, Kemin Nutrisurance, Italy; Ewa Fuller, Kemin Industries/Nutrisurance, USA

Over the past years a clear trend in the pet food business has been emerging where manufacturers are reducing the use of synthetic molecules and focusing more on natural antioxidants. Since only tocopherols are registered as natural antioxidants in Europe, a deep understanding of the mode of action of the different tocopherol homologues is required. In this study, the antioxidant activity of the four homologues (alpha, beta, gamma and delta) was investigated.

Tocopherols from different plant sources (soybean, sunflower, rapeseed) were analyzed with respect to their profile of alpha, beta, gamma, and delta homologues. A significant difference in the content of the homologues was observed having $\alpha$-rich tocopherols from sunflower and $\delta$-rich tocopherols from soybean and rapeseed plant sources, respectively.

The tocopherols were blended into fresh animal fats at dosages from 500 ppm to 2000 ppm. All samples including a negative control without tocopherols were monitored using the Oxidative Stability Instrument (OSI) at elevated temperatures. A continuous flow of air bubbling through the liquid samples accelerates the oxidation process in this device.

A direct relation between the induction point as an indicator for the stability against oxidation and the corresponding homologue profile of the sample was identified. In fish oil (figure), the control (blue) and the samples stabilized with tocopherols from sunflower (red; rich in $\alpha$) showed relatively short induction periods indicating a fast oxidation. The samples with tocopherol coming from soybean (orange) and rapeseed (purple) oil showed significantly longer induction periods.

**Evaluation of Oxidative Stability of Full-fat Soybean Flour Stored Under Accelerated Conditions as Influenced by Traditional Processing Methods.** Ece, Gulkirpik, University of Illinois at Urbana Champaign; USA; Marco Toc, University of Illinois at Urbana Champaign, USA; Richard A. Atuna, University for Development, Studies, Ghana; Francis K. Amagloh, University for Development Studies, Ghana; Juan E. Andrade Laborde, University of Florida, USA

Justification: In Sub-Saharan Africa, full-fat soybean flour (FFSF) is a commercially used, low-cost ingredient to complement the protein content of cereal-based blends. Its high polyunsaturated oil content is prone to oxidation in most tropical climates, however, limiting its use in food applications.

Objective. To examine the effect of pretreatment of soybeans using malting (80%, form hypocotyl), soaking (18h and 24h), roasting (110–120oC, 10 min) and dehulling, on the oxidative stability of FFSF for 12 weeks under room (23 $\pm$ 0.1oC, 25.7 $\pm$ 5.4% relative humidity, RH) and accelerated conditions (45 $\pm$ 0.1oC, 80–82% RH).

Methods: Two batches of FFSF samples were prepared in Ghana one of which was dehulled. Raw samples were the control. Samples (25g) were stored in flat polypropylene heat-sealed bags before placing under either condition. Samples were collected after 0, 4, 8, 12 weeks. Acid value (AV), peroxide value (PV), p-anisidine value (pAV), lipoxygenase activity (LOX), color, moisture, and water activity were measured using standard methods. A three-way Mixed ANOVA was used to evaluate the effect of pretreatment, condition, and time on physical-chemical parameters.

Results: Pretreatment, condition*time, and pretreatment*condition*time had a significant impact on AV, PV, pAV, LOX, color values of all samples ($p < 0.001$). Accelerated conditions promoted oxidation in FFSF regardless of the pretreatment method. Roasted dehulled treatment resulted in the highest increase in AV and pAV in both storage conditions ($p < 0.05$). Raw samples had the highest LOX activity while roasted and malted ones had the lowest. Under accelerated conditions, LOX activity was almost absent in all samples by the end of 12 weeks. Malting and soaking processing methods significantly reduced oxidation ($p < 0.05$) of FFSF.

Significance: Traditional processing methods such as malting and soaking can improve oxidative stability and therefore potentially expand the shelf life of FFSF. Roasting promotes oxidation despite its common use.

**Exposure of squalene hydroperoxides to photons causes the formation of unique squalene cyclic peroxides via a chain reaction mechanism detected on the human skin and causing harm to skin cells (HaCaT).** Saoussane Khalifa, Tohoku University, Japan; Shunji Kato, Tohoku University, Japan; Masaru Enomoto, Tohoku University, USA; Yurika Otoki, Tohoku University, Japan; Takahiro Eitsuka, Tohoku University, USA; Kiyotaka Nakagawa, Tohoku University, USA

Squalene (SQ) (Fig.1) is one of the most abundant skin lipids that plays an overall protective role on the skin. However, it has been demonstrated that SQ on the skin can be readily oxidized to form six monohydroperoxide isomers (SQ-OOH) (Fig.1) as the primary oxidation products upon reaction with singlet oxygen. As hydroperoxides are considered to be rather unstable, the secondary oxidation products deriving from their further modification under oxidative conditions are still unclear. In this study, we focused on the structural elucidation of unique squalene cyclic peroxides deriving from SQ-OOH isomers, the mechanism involved in their formation, their detection on the human skin, and the damage that they can cause to skin cells (HaCaT) in comparison to SQ-OOH.

SQ-OOH isomers were synthesized by singlet oxygen oxidation then purified via multiple steps. The secondary oxidation products were synthesized by subjecting SQ-OOH isomers to a second oxidation, the mechanism generating these compounds was studied by carrying out the second oxidation in different conditions. Their structures were studied using LC-MS/MS, NMR and derivatization reactions. Human skin surface lipids were sampled using acetone-soaked cotton swabs, further extracted and purified, then analyzed using LC-MS/MS. HaCaT cells were used to assess the effect of the primary and secondary oxidation products on the skin.

The secondary oxidation products in question were revealed to be cyclic peroxides deriving from individual SQ-OOH isomers upon their exposure to photons in the presence of ground state molecular oxygen. The presence of the cyclic peroxides derived from 6-OOH-SQ and 10-OOH-SQ was confirmed on the skin, they were also detected in the medium after incubation of 6-OOH-SQ and 10-OOH-SQ with HaCaT cells. This was also linked to a high cytotoxic effect observed with 6-OOH-SQ, 10-OOH-SQ, and even higher in the case where the pure secondary oxidation product deriving from 6-OOH-SQ was used.

**Furan fatty acid profiles provide insights into quality of soy products.** Franziska Müller, University of Hohenheim, Germany; Melanie Hogg, Ulm University, Germany; Walter Vetter, University of Hohenheim, Germany

Furan fatty acids (FuFAs) are minor fatty acids having a characteristic furan moiety within their alkyl chain. The methylation degree in the β-/β'-position of this furan ring influences the properties and indicates the origin of FuFAs. Namely, mono-/dimethylated FuFAs (M-/D-FuFA) are natural products that are biosynthesized by plants and bacteria. By contrast, 3,4-nonmethylated FuFAs (N-FuFAs) are rarely described secondary oxidation products of unsaturated fatty acids. The desired labile D-/M-FuFAs have excellent antioxidative and radical scavenging properties, whereas N-FuFAs are more stable but poor antioxidants.

Freshly harvested soybeans are known to contain comparably high amounts of three D-/M-FuFA. Specifically, predominance of the two D-FuFAs 11-(3,4-dimethyl-5-pentylfuran-2-yl)-undecanoic acid (11D5) and 9-(3,4-dimethyl-5-pentylfuran-2-yl)-nonanoic acid (9D5) is accompanied with the M-FuFA 9-(3-methyl-5-pentylfuran-2-yl)-nonanoic acid (9M5).

The influence of processing on the content of 11D5, 9D5, 9M5 and N-FuFAs was studied in freshly harvested soybeans (n = 4) and soy products of different processing stages (n = 22) purchased at German retailers. Analysis was based on three steps, i.e., (i) conversion of all fatty acids into methyl esters (ME), (ii) separation of FuFA-ME from conventional FAMEs by silver ion chromatography, and (iii) gas chromatography with mass spectrometry (GC/MS) analysis.

D-/M-FuFA levels decreased with increasing processing of the product. Since stabilities of FuFAs are decreasing in the order N- > >M- >D-FuFAs, the FuFA pattern was additionally changed in favour of 9M5. In addition, 8-(5-hexylfuran-2-yl)-octanoic acid (8F6) and partly lower amounts of 7-(5-heptylfuran-2-yl)-heptanoic acid (7F7) were detected in all processed products but not in freshly picked soybeans. These findings seemed to be generally valid because the samples were processed by different manufacturers. Hence, FuFA profiles are suggested as indicators for careful storage and processing of soybeans. Premium products should be characterized by a clear dominance of D-FuFAs over M-FuFAs (natural pattern) and the virtual absence of N-FuFAs (oxidation products).

**Hexane Extraction of Meat Meal for Peroxide Value Titrimetric Analysis.** Amanda Trainer, Hanna Instruments, USA

AOCS Cd 8b-90 for Peroxide Value (PV) details the titration procedure for oil and fat samples; however, it does not include pre-analysis oil extraction if a complex matrix is to be analyzed. Such products, such as meat meal, may instead be tested by other means, such as the SafTest, which for some customers is insufficient in regards to cost, accuracy, and repeatability. There is a discernible need for a more absolute method and formalized procedure for testing such products with a complex matrix. Presented is a proposed extraction method for meat meal to be used in dry pet food.

Meat meal is mixed with n-hexane to extract the oil and fat into the solvent; a ratio of 1:1 to 1:2 is recommended to ensure sufficient extraction and minimize both time and reagent cost.

The resulting mixture is filtered to separate particulate matter from the solvent, where, due to the limitations of gravity filtration, it is highly recommended to utilize vacuum filtration, when possible, in order to minimize filtration time and maximize effectiveness; it is vital that negligible particulates remain in the filtrate to ensure accuracy of the titration results.

The solvent is evaporated off to yield an oil/fat residue that is subsequently massed and analyzed per AOCS Cd 8b-90. Due to known effects of air and light on PV, it is highly recommended to utilize a rotary evaporator as opposed to natural evaporation as the estimated time of evaporation is significantly reduced from several hours to 15 minutes; it is vital that negligible solvent remains in the residue to ensure accuracy of the titration results.

**Improving the oxidative stability of side-streams from cod filleting by antioxidant dipping for increased utilization.** Ann-Dorit Sørensen, National Food Institute (DTU Food), Denmark; Haizhou Wu, Chalmers University of Technology, Sweden; Grethe Hyldig, Technical University of Denmark, Denmark; Ole Mejlholm, Royal Greenland Seafood A/S, Denmark; Niels Bøknæs, Royal Greenland Seafood A/S, Denmark; Ingrid Undeland, Chalmers University of Technology, Sweden; Charlotte Jacobsen, Technical University of Denmark, Denmark

A growing interest for sustainable food production and increased utilization of existing food raw materials, hereunder side-streams, have led to increased research in this area. In the cod filleting industry up to 60% of the raw materials end up as side-streams (guts, heads and frames). Currently, the majority of these side-streams ends up as low value products for feed. To increase utilization for food applications, new preservation solutions are needed to maintain a high quality of the side-stream before further processing.

The aim of this study was to evaluate the effect of antioxidant dipping of cod side-streams on their oxidative stability during subsequent storage. The following treatments were evaluated: no dipping, dipping in 0.9% NaCl, dipping in 2% Duralox MANC (commercial rosemary preparation fortified with ascorbic acid, α-tocopherol and citric acid), or dipping in 0.05% and 0.2% lipophilic rosemary extract having a carnosic acid content 15 times higher

than that in Duralox-MANC. After antioxidant dipping, the side-streams were drained, minced to obtain homogeneous samples and subjected to short- term storage (7 days, 5oC). The effect of the antioxidant treatment was evaluated by measuring peroxide value (PV), TBA-reactive substances (TBARS) and sensory during storage.
Results show a positive effect of the antioxidant dipping on the oxidative stability of cod side-streams (guts, heads and frames). The most efficient antioxidants in all three side-streams were Duralox MANC and Rosemary extract (0.2%). For guts, Duralox MANC inhibited the formation of PV throughout the whole storage period and TBARS were inhibited for 4 days. Rosemary extract (0.2%) also inhibited the formation of PV for 7 days, however, the inhibition of TBARS was only 2 days. For the non-dipped guts, PV and TBARS already increased after 1 day of storage. The same pattern was observed for frames, however, the overall concentration of TBARS was much lower.

**Lipase-Assisted Production of 1-o-galloylglycerol: Characterization and its Antioxidant Properties.** Siyu Zhang, University of Georgia, USA; Casimir Akoh, University of Georgia, USA
Galloylglycerol (GG) is a water-soluble derivative of gallic acid (GA), and a phenolic secondary metabolite of many plants. Conventionally, GG is synthesized by chemical esterifications and transesterifications with low yield and the reaction conditions are not suitable for producing bioactive compounds to be used in foods. In this work, GG was synthesized by lipase-assisted glycerolysis of propyl gallate (PG) using an immobilized commercially available food-grade *Candida antarctica*; lipase B (Lipozyme®p> 435). The reaction was performed in a 100-mL double-layer jacketed glass reactor equipped with a circulating water bath, stirred with a PTFE anchor paddle stirring rod, and using glycerol as both the reactant and solvent. Under optimal conditions (55 °C, 25:1 glycerol:PG substrate molar ratio, 120 h, and 23.8% enzyme load relative to the total weight of the substrates) a yield of 76.9% ± 1.2% was obtained. The antioxidant properties, water solubility, n-octanol/water partition coefficient, and molar extinction coefficients of GG were determined after being separated from the reaction mixture using liquid-liquid extraction. The water solubility and hydrophilicity (measured by n-octanol/water partition coefficient) of GG were significantly higher than those of GA and PG. The antioxidant properties, measured by the ferric reducing antioxidant power (FRAP) and hydrogen peroxide ($H_2O_2$) scavenging assays, showed that GG exhibited the highest scavenging capacity (GG > GA > PG). From the results of the 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) assays, GG and GA exhibited greater scavenging capacity than PG (GG = GA > PG). GG may be used as a water-soluble antioxidant in food and cosmetic applications.

**New insight into complex lipid oxidation pathways from quantum chemistry.** Thomas Mustard, Schrodinger, USA; Jeffrey Sanders, Schrodinger, USA
Lipids are frequently used in food products, cleaning supplies and cosmetics for their advantageous properties. Degradation of lipids via oxidation can lead to shorter shelf lives, off-flavor tastes, and deterioration of product properties. Despite common oils being used in different industries, the dominant degradation pathways are significantly different depending on the specific application. Knowledge of pathway degradation, especially when competing mechanisms exist could help guide better selection of oils for particular products. Using quantum chemistry methods, we explore different degradation pathways with the intent to understand the energetics of reactions schemes. Prediction of key properties like bond dissociation energies rate constants for competing reaction pathways are also addressed and its applicability to product design.

**Revised DNPH analysis of lipid carbonyls: reaction conditions for accurate quantitation and HPLC conditions for separation of individual carbonyls.** Morgan Kandrac, Rutgers University, USA; Karen Schaich, Rutgers University, USA
Although dinitrophenylhydrazine (DNPH) reaction has been used for quantitation of non-volatile carbonyl products of lipids oxidation for decades, the assay has not been developed for routine use due to issues with reactivity and instability. Increased recognition that products from multiple oxidation pathways other than hydroperoxides must be tracked for accurate assessment of lipid oxidation now provides a new driving force for reconsidering and revising this assay for accuracy, sensitivity, and ease of use. This paper provides evidence that traditional reaction conditions use excess acid that protonates DNPH to non-reactive DNPH2, decreasing detection and quantitation of carbonyls. Reaction pH was investigated on both a mixture of carbonyl standards and neat, oxidized methyl esters; hydrazones were separated using RP-HPLC with response measured by diode array at 360 nm. Increasing the reaction pH > 2.5 allows stoichiometric levels of DNPH to be used and provides complete reaction with carbonyls. Most significantly, reaction at the standard pH 1 resulted in 50% less total peak area than at pH 2.5 with oxidized methyl oleate. Optical quantitation of the reaction in solution Is difficult but hydrazones can be separated from unreacted DNPH by HPLC, preventing excess reaction and providing an additional advantage of identification and quantitation of individual carbonyl products, which is very useful in mechanism studies. Either reversed phase or normal phase HPLC can be used.

**The method of removing FFA, Pv, P-anv, and TPM from industrial frying oil application.** Joby Ulahanan, Crystal Filtration Co., USA

Objective/Hypothesis: There are different types of vegetable oil and animal fat used in frying oil application. The finished products range from other kinds of snack foods.

One of the significant problems facing in snack foods industries is oil degradation due to increased free fatty acids, peroxide, P-anv and TPM, and other undesired components. This article provides an overview of how to remove FFA, PV, P-and, and TPM in snack foods industrial frying oil application.

Method Used: The oil quality parameters like free fatty acis, peroxide, anisidine, and TPM, are measured using the CDR Foodlab analysis system.

Results: Vegetable oil and edible oil samples were collected from a different industrial application. Before the particular grade adsorbent treatment, free fatty acid and peroxide, P-anv, and TPM of oil samples were measured. The oil sample was heated in a Lab-scale pilot R&D set up.

A particular grade adsorbent used in these trials was up to 2% of cooking oil weight. After 10 minutes of reaction & mixing time, started filtration. Then measured free fatty acids and peroxide, P-anv, and TPM.

The free fatty acids reduced 50–75%, peroxide decreased 5–35%, P-and reduced 4–8%, and TPM value 5–10% for the selected oil samples.

Conclusion: A special grade Adsorbent treatment in snack foods frying oil application is economical and extends the life of cooking oil and increase final product flavor and shelf life.

**Underrated potential of chocolate by-product, cocoa bean shells, as a nutrient-rich and bioactive food ingredient.** Kathleen Fung, University of Guelph, Canada; Fernanda Peyronel, University of Guelph, Canada

The increased consumer demand for chocolate generates a problem for cocoa waste management, with more than 650 thousand tonnes of cocoa bean shells (CBSs) produced yearly. Minimizing waste by transforming this underutilized by-product into a resource for other purposes is one of the main principles of a circular economy. Typically discarded, CBSs are a nutrient-rich food ingredient with untapped potential. Most importantly, CBSs contains significant amounts of useful dietary fibres and polyphenols. Flavonoids, such as epicatechins and catechins, and anthocyanins are the major phenolic compounds present in cocoa beans. Dietary fibres and polyphenols impart many scientifically proven health benefits, including improving gut and heart health, as well as reducing oxidative damage. Despite the nutrient-rich compounds present in CBSs, there have been limited studies carried out on adding whole CBSs into different food matrices. Monitoring and mitigating food safety hazards in CBSs, such as mycotoxins, heavy metals, and microorganisms, ensure food safety and are essential for quality assurance. Extracting and repurposing CBSs' bioactives are the focus of existing CBSs studies. However, the food waste problem persists because the CBSs residue remains, and the extraction methods generate more waste. In this work, we show the main benefits of recycling a by-product, CBSs as a whole, with an emphasis on polyphenols. We also discuss various strategies to mitigate CBSs food safety hazards. Furthermore, our findings can help reduce the global food waste problem and introduce the novel fibre-rich antioxidant source for food enrichment in various food matrices.

**Using adsorbents to sustain the oxidation stability of biodiesel and its blends.** Jerome Desire Aliebakaa Kpan, Coburg University of Applied Sciences, Germany; Jürgen Krahl, OWL University of Applies Sciences and Arts, Germany, Germany

Fuels used in plug-in hybrid vehicles and standby power plants, stay longer in the tank possibly lasting a full year or more and get deteriorated. It is important to maintain the fuel quality over the entire period that it stays in the tank. While others periodically burn up the old fuel in the tank to keep the fuel stable, some automatically shift from electric to hybrid mode to burn of fuel at defined periods such as every 90 days. This study investigates the impact of adsorbents on the oxidation stability of biodiesel and its blends. Biodiesel fuels contain significant amounts of unsaturated fatty acid esters and therefore, are prone to oxidative degradation which deteriorate the quality of the fuel. Fuels in plug-in hybrid vehicles especially biodiesel and its blends over a storage period of time deteriorate. Diesel fuel (EN590) blended with biodiesel was thermo oxidatively aged using the Rancimat. The fuel mixture was treated with adsorbents magnesium aluminum hydroxycarbonate and 1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl) benzene. The adsorbents offered an effective performance by significantly increasing the oxidation stability of the mixture by a factor of 4. There was about 90 % reduction in acid value. The adsorbents demonstrated high effectiveness in oxidative stabilization of biodiesel and its blends.

**Volatile organic compounds of rancid vegetable oils subjected to prolonged thermal treatment.** Amelia Najwa Ahmad Hairi, Sime Darby Plantation Research Sdn Bhd, Malaysia; Nur Azwani Ab Karim, Sime Darby Plantation Research Sdn Bhd, Malaysia; Norliza Saparin, Sime Darby Plantation Research, Malaysia; Ahmadilfitri Md Noor, Sime Darby Plantation Research Sdn Bhd, Malaysia

Volatile compounds of palm oil, palm olein, sunflower oil, soybean oil and olive oil subjected to prolonged

thermal treatment were analyzed. The oil samples were stored at 90 °C for 8 days and the samples were collected at day 4 and day 8 for analysis. The volatiles emitted were determined by a static headspace gas chromatography - mass spectrometry (GCMS) system. The results after thermal treatment in sunflower and soybean oils showed inconsistent levels of emitted volatiles whereas palm oil, palm olein and olive oil displayed increasing volatile levels as storage days increased. Several major volatile compounds were identified with hexanal as the major constituent ranging from 22% to 51% of the overall volatiles. It was found that the volatile compounds were closely related to the overall oxidation levels analyzed by UV spectrometer.

***Abstracts for the following Lipid Oxidation and Quality presentations were not provided.***

**Industry Updates II Presentation.** Matthew Russell, The Tintometer Limited (Lovibond), United Kingdom

**Process Automation Using FT-NIR Spectroscopy: Applications in The Oils and Oilseeds Industry.** Christopher Clark, Bruker, USA

**Contaminants in Vegetable Oils.** Norliza Saparin, Sime Darby Plantation Research, Malaysia; Syed Mohd Hadi Syed Hilmi, Sime Darby Plantation Research Sdn. Bhd., Malaysia; Rahmat Ngteni, Sime Darby Plantation Research Sdn. Bhd., Malaysia

## Phospholipids Division

*Vice Chair: Ozan Ciftci, University of Nebraska, USA*

### General Aspects of Phospholipids

*Chairs Tao Fei, University of Tennessee, USA; Swapnil Jadhav, ADM, USA; Junsi Yang, University of Nebraska-Lincoln, USA*

**Characterization and Anti-microbial Activity of Castor Oil-based Microemulsion.** Chintya Gunarto, National Taiwan University of Science and Technology, Taiwan (Republic of China); Alchris Woo Go, National Taiwan University of Science and Technology, Taiwan (Republic of China); Artik Elisa Angkawijaya, National Taiwan University of Science and Technology, Taiwan (Republic of China); Yi-Hsu Ju, National Taiwan University of Science and Technology, Taiwan (Republic of China)

Castor oil (CO), extracted from castor seeds, is widely used in the chemical industry, fuel, and pharmaceutical applications. It could be adopted for use as the oil phase in the formulation of microemulsion (ME). A microemulsion is a unique mixture consisting of an oil, and an aqueous phase, stabilized by surfactant and/or co-surfactant to reduce the interfacial tension to form a one-phase system. Various formulations of CO-based MEs were characterized in this work, including their hydrodynamic diameter, polydispersity, and zeta potential. In addition, the anti-microbial activity of CO-based MEs with tween 80 as the surfactant and ethanol as the co-surfactant were investigated. Formulations with higher CO in the ME resulted in higher microbial inhibition and less water in the ME were found to be more stable based on the determined zeta potentials. The ME formulation with 15 wt.% CO, 75 wt.% surfactant mixture and 10 wt. % DI water resulted in the highest anti-microbial activity, inhibiting the growth of both Escherichia coli and Staphylococcus aureus as gram-negative and gram-positive bacteria, respectively. From the current results, CO-based ME formulations may potentially be adopted for use in drug delivery systems for hydrophobic drugs, especially for topical applications.

**Efficient Expression of Phospholipase D and Its Application in Enzymatic Modification of Phospholipids.** Xiangzhao Mao, Ocean University of China, China (People's Republic)

Phospholipids are widely being used in food, medicine, cosmetics and other fields due to their unique chemical structures as well as healthcare functions. Studies have shown that the polar head groups in the molecular structure of phospholipids have a significant impact on their nutritional functions and performance. Phospholipase D (PLD, EC 3.1.4.4) can catalyze the transphosphatidylation reaction to synthesize phospholipids with specific head groups; this method not only has mild reaction conditions and high product purity, but also can obtain rare phospholipids with important applications. However, the existing expression level, enzyme activity and selectivity of PLD limit their industrial application. Here, cell surface display and DNA shuffling technology were used for the screening and preparation of PLDs with high transphosphatidylation activity. The best performing recombinant enzyme in transphosphatidylation reaction was Recom-34, the conversion rate was 80.3% and a 3.24-fold increase compared to the parental enzyme. On this basis, we constructed a PLD expression and secretion system in Bacillus subtilis and developed an environmentally friendly reaction system for enzymatic synthesis of Phosphatidylserine (PS) and phosphatidyl-glycosides. After optimizing fermentation conditions, the enzyme activity of the culture medium was 0.35 U/mL. When using cyclopentyl methyl ether as the organic phase, the final conversion rate of PS reached 96.0% However, in the reaction process of phosphatidylcholine with glucose, glucosamine and N-acetylglucosamine, it showed substrate selectivity. Molecular docking technology was adopted to reveal

which amino acids played an important role in those process for synthesis of these compounds. What's more, this study also provides a reference for screening ideal biocatalysts, brakes through the limitation of the traditional technology and lays a foundation for further research on lipids intake.

**Milk Polar Lipids: Potential for Management of Dysfunctional Lipid Metabolism, Gut Dysbiosis, and Chronic Inflammation.** Christopher Blesso, University of Connecticut, USA

Milk fat is encased in a tri-layer milk fat globule membrane, which contains a variety of polar lipids and proteins. Milk polar lipids include phospholipids and sphingolipids, which together account for up to 1% of total milk lipids. The surfactant properties of milk polar lipids are important for dairy products and recent evidence also suggests these lipids can influence nutrition and health. This presentation will focus on the potential of milk polar lipids in protecting against dysfunctional lipid metabolism and inflammation in the context of metabolic disease. Evidence of milk polar lipids in modulating the gut microbiome will also be discussed.

**Polar Lipids in Human Milk and Its Potential Functions.** Wei Wei, Jiangnan University, China (People's Republic); Xingguo Wang, Jiangnan University, China (People's Republic)

Human milk fat is a mixture dominated by complex natural lipids. It contains more than 200 fatty acids and 400 triacylglycerols which account for ~98% of human milk. There are also numerous complex lipids including glycerophospholipids, sphingolipids, sterol lipids, which are all arranged in distinctive molecular configurations and structures. Phospholipids were quantified by 31P NMR and its molecular species were identified using UPLC-Q-TOF-MS. The gangliosides and cholesterol were analyzed using UPLC-Q-TOF-MS and GC-MS, respectively. The results indicated that polar lipids in human milk are complex with more than 160 polar lipids were identified. There are some common polar lipids in mammalian milk, while human milk contains some special characteristics of polar lipids for example the high amount of sphingomyelin. The observation of polar lipids using CLSM showed that they all located in the membrane of human milk fat globules. However, significant differences were observed between the size & structure of human milk and infant formula as well as other dairy products. Recent clinical trials reveal that MFGM supplements which mainly bovine-derived protein and phospholipid mixtures may help the optimal brain development of infants. The optimization of polar lipids obtained from the different dairy processing needs further study.

**Switchable Solvents – a Novel Extraction Method for Milk Phospholipids from Dairy By-product Stream.** Kaavya Rathnakumar, South Dakota State University, USA; Sergio Martinez-Monteagudo, New Mexico State University, USA

Milk phospholipids (PLs) are recognized for their health benefits. Currently, extraction of the PLs fraction from concentrated streams results in low efficiency, and it involves subsequent solvent separation and lipid recovery. This research aims at developing a PLs extraction process from dairy byproducts using switchable solvents. The new generation of solvents are smart and can be reversibly switched between a hydrophobic and hydrophilic form by simply bubbling or removing CO2 and can be reused. This novel approach requires neither distillation nor the use of volatile, flammable or chlorinated solvents. Different dairy by-products streams were used to evaluate the feasibility of switchable solvent extraction. A tertiary anime (N, N-dimethyl cyclohexylamine, CyNMe2) was used as a switchable solvent. The recovered PLs were quantified by thin-layer chromatography and HPLC. For comparison, PLs were also extracted using the Folch method (FM) and the Mojonnier method (MM). Statistical analysis was carried out to identify the significance of different process parameters. The extraction efficiency of CyNMe2 ranged from 0.33–99%, depending on the type of byproduct. Remarkably, the CyNMe2 extracted up to 99% of the PLs directly from buttermilk. Improvements in the extraction efficiency of PLs from B-serum was done by the effect of acoustic intensity ($44.56 \pm 3.47$ W cm-2) before CyNMe2 extraction (1/12mL) ratio recovered $69.07 \pm 0.11$% of PLs, a 9.8 fold increment than the samples without ultrasound-pretreatment. The recovered fraction of PLs mainly comprised of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%). Optimization was done with maximum desirability of 0.982 and the model was significant. The model was significant for all three responses and the co-efficient of determination(R2) were 0.9825, 0.8164 and 0.7060 for 3, 10, 18 h. For CyNMe2, scanning and confocal microscopy-images, particle size, and gel electrophoresis revealed great disruption of the protein matrix, releasing the PLs into the aqueous medium.

**The Omega-3 Index—Why Red Cell Phospholipid Fatty Acid Levels Matter and Not Fatty Acid Intake.** Clemens Von Schacky, Omegametrix, Germany

The Omega-3 Index is % of EPA&DHA in erythrocyte phospholipid fatty acids. With >300 publications, the original, highly standardized analytical method "HS-Omega-3 Index®" has the largest scientific database for fatty acid analyses. Human life with an Omega-3 Index < 2% has not been found, making EPA&DHA essential for survival. Moreover, all clinical events and effects studied so far correlated closely with the Omega-3 Index, much less with intake. In contrast, effects of EPA&DHA were investigated with scientific methods developed for drugs: recruiting participants

irrespective of baseline levels; comparing the effects of a single fixed dose with placebo. Therefore, design and results of many trials need to be reconsidered before including them into meta-analyses, and results of many previous meta-analyses are invalidated.
In epidemiologic studies, the Omega-3 Index correlated inversely with total mortality, cardiovascular events, premature birth, major depression, impaired cognition, and other frequent health issues. Intervention trials found improvements in these clinical parameters to correlate with the Omega-3 Index or other levels of EPA&DHA. Evaluating results of the large REDUCE-It trial based on levels reached, reductions in total mortality (–40%) or stroke (–50%) were larger than evaluating the same trial, comparing verum with placebo: total mortality –13%, stroke –28%. The same was true for all other endpoints studied. Thus, results of epidemiologic studies and intervention trials became qualitatively and quantitatively comparable, which was never the case when comparing data on intake.
In many countries in the world, the mean Omega-3 Index of the respective populations is (far) below the target range of 8–11%. National Statistics Canada has representatively determined a mean HS-Omega-3 Index of 4.5% in the Canadian population. Alpha-linolenic fails to increase the Omega-3 Index, but according to regulatory authorities, EPA&DHA are safe and well tolerated up to 5 g/day (EFSA) or 3 g/day (FDA).

## Novel Phospholipids; Pharmaceutical, Functional and Edible Applications

*Chairs Ernesto Hernandez, Advanced Lipid Consultants USA, Mabel Tomas, CIDCA-UNLP, Argentina*

**Application of Modified Sunflower Lecithin in the Development of Delivery Systems of Chia Seed Oil.** Mabel Tomas CIDCA-UNLP, Argentina; Luciana Julio, CIDCA-CONICET UNLP, Argentina; Claudia Copado, CIDCA-CONICET UNLP, Argentina; Bernd Diehl, Spectral Service, Germany; Vanesa Ixtaina, CIDCA-CONICET UNLP, Argentina
The development of delivery systems like emulsions is one of the strategies for preserving and enriching food matrices with functional lipids. Chia seed oil is the vegetable source with the highest level of a-linolenic acid (~ 65%) and important level of PUFAs (~80%) being very susceptible to lipid oxidation. Modified sunflower lecithins have good O/W emulsifying properties. Conventional or multilayer O/W emulsions with deoiled (DL) or hydrolyzed sunflower lecithin (HL) and chia seed oil could be considered for that purpose. Multilayer emulsions (5% chia oil) with DL or HL at pH 5 (anionic character) were obtained by high pressure homogenization and characterized. The application of an electrostatic deposition layer-by-layer technique with the addition of chitosan and chia mucilage allowed to obtain the bi and trilayer systems, respectively. The three-layer emulsions differed significantly from the other systems, recording the highest values of D3,2 and Span. Monolayer emulsions with HL presented larger sizes than emulsions with DL. Trilayer systems were more stable, due to the greater thickness of the interfacial membrane than the monolayer ones and the functional properties of chia mucilage (high level of water absorption and retention capacities). These results represent interesting information and constitute a good alternative to protect and deliver chia seed oil into functional foods.

**Composition and Emulsifying Properties of Water and Enzyme Degummed Camelina Sativa Lecithin.** Henok Belayneh, Terviva Bioenergy, Inc., USA; Ozan Ciftci, University of Nebraska-Lincoln, USA
Camelina seed is a promising source of omega-3 oil. In addition to its omega-3 oil content, it is a new source for lecithin. However, there is little information on the chemical composition of camelina seed lecithin; therefore, the objective of this study was to investigate the chemical composition and emulsifying properties of lecithin separated from camelina seed oil by water and enzymatic degumming. Both water and enzyme degumming yielded phosphatidylinositol (PI)-rich lecithins, and contents were 37.8 and 25.2 % (w/w), respectively. Lecithin obtained by enzymatic degumming yielded more lysophospholipids compared to water degumming. Enzyme-degummed camelina lecithin contained more saturated fatty acids compared to water-degummed one. Emulsions prepared with enzyme degummed camelina lecithin formed highest stability emulsions when deionized water was used as the aqueous phase (original pH); however, emulsions were less stable compared to the emulsion stabilized with soy lecithin at pH=7.5. Results showed that camelina seed lecithin is an alternative PI-rich emulsifier for various food applications.

**New Modified Phospholipid for Stabilization of Beverages.** Ernesto Hernandez, Advanced Lipid Consultants, USA
New trends in health-oriented beverages include a preference to use ingredients that are all natural. So there is a continuous need for the development of emulsifiers and weighting agents that meet this requirement. Phospholipids are already widely utilized as natural emulsifies in foods and beverages and now are also widely used for their health and bioactive properties.
We have developed modified phospholipid from blends of vegetable oil lecithins and higher specific gravity sugars in the preparation and stabilization of emulsions for beverages. The main objectives of this work include the use of new lecithin emulsifier blends to prevent

creaming, coalescing and phase separation of suspended lipid globules and extend their applications to dairy and protein drinks as well as carbonated beverages. The methods of emulsion preparation included high shear mixing and high-pressure homogenization.
These oil in water emulsions were manufactured using modified lecithins, higher specific gravity sugars, hydrocolloids, and natural and synthetic weighting agents. The resulting emulsions and beverages were analyzed for emulsion stability, suspended particle distribution, organoleptic quality and shelf life. The new 'weighted' lecithins were able to prevent creaming, coalescing and phase separation in the beverages prepared. And the sugars used with the phospholipids effectively prevented phase separation and balanced the specific gravity of the dispersed and continuous phases. The particle size distribution of the suspended lipid globules in the beverages prepared ranged between 1 and 10 microns and shelf life was comparable to commercial products. The modified lecithins were effective in stabilizing emulsions and replace weighting agents such as ester gum and sucrose ester isobutyrate, commonly used in commercial beverages.

**Recent Advances in the Characterization of Milk Fat Globule Membrane and How It Affects Brain Function.** Sandra Einerhand, Einerhand Science & Innovation, Netherlands
The brain is a lipid rich organ, and complex lipids such as phospholipids and gangliosides, comprise up to 35% of the total lipids in the brain. These complex lipids are critically important for brain function. Milk fat droplets are covered by the milk fat globule membrane (MFGM) containing complex lipids. Over the past decade, MFGM has been extensively studied. Human intervention trials, especially in children but also in adults, have shown that MFGM has effects on cognitive function, mood and behavior. However, thus far several different commercially available MFGM sources have been used in studies. From recent omics data it is clear that these MFGM preparations vary in composition. Here, we will provide an overview of the clinical studies in adults and children and correlate those findings with the latest omics data revealing details on complex lipid composition of the different MFGM sources. This will help to unravel which complex lipid components in MFGM are the (most) biological active ones.

**Use of Phospholipid-containing Films to Enhance Supersaturation of Hydrophobic Drugs for Improved Drug Release in Oral Drug Delivery.** Shawn Wettig, University of Waterloo, Canada; Jiahao Huang, University of Waterloo, Canada
Amorphous solid dispersion (ASD) formulations are widely used to deliver poorly soluble drugs by dissolution enhancement through achieving supersaturation in aqueous environment. Liquid-liquid phase separation (LLPS) can occur for a supersaturated solution and result in drug nucleation and crystallization that ultimately decreases the effectiveness of ASDs. The purpose of this study was to use phospholipids as functional excipients to delay LLPS and maintain ASD supersaturation.
A BCS class II drug indomethacin (IDM) was used as model drug. The LLPS profiles of IDM supersaturated solution in the presence of excipients was characterized by a double-wavelength UV extinction method. The crystallization rates of IDM in the presence of excipients were assessed by a solvent-shift test under non-sink dissolution conditions. A phospholipid-polymer binary system was used as the ASD carrier and film forming base to test the kinetic-solubility profiles of IDM under non-sink dissolution conditions. Phospholipid was combined with common polymeric ASD carriers (polyvinylpyrrolidone and ethyl cellulose) to form edible film formulations.
The addition of phospholipid significantly delayed the LLPS of IDM from 52.9 μg/mL in pure water to 171 μg/mL in 360 μg/mL phospholipid solution, prolonging the existence of drug-rich nanodroplets upon LLPS formation. The IDM supersaturation was effectively maintained by phospholipid at the initial precipitation stage (< 60 min) with a non-monotonic change in drug crystallization rate under non-sink condition, corresponding to LLPS evolution. IDM ASD formulated by different phospholipid-polymer compositions showed adjustable dissolution parameters such as Tmax, Cmax, supersaturation degree and duration, etc. Adjustable drug release profiles could also be achieved by stacking phospholipid-containing films following different geometric models, providing a simple and suitable alternative for pharmacy compounding for hydrophobic drugs.

## Sustainable Processing and Fractionation for Novel Phospholipids - Including Milkfat, Marine and Plant-Based Phospholipids

*Chairs Ozan Ciftci, University of Nebraska-Lincoln, USA; Laurent Bazinet, Laval University, Canada*

**Green Processes for Phospholipid Recovery and Fractionation.** Nurhan Dunford, Oklahoma State University, USA
Phospholipids (PL) are a group of molecules having both hydrophilic and hydrophobic head groups. They are important part of plant and animal cells and play role in many biological functions in human body. Various health benefits of PL naturally present in seed oils have been reported in scientific literature. They are widely used in diverse applications in food, pharmaceutical and cosmetics industries.
PL are extracted along with other lipids during conventional solvent extraction of oils from oilseeds. However,

these compounds are separated from crude vegetable oils during a refining process referred to as degumming. They end up in the processing byproduct stream, commonly known as gums. Commercial products containing PL are usually recovered from the byproducts.

Conventional lecithin (a mixture of PL) production techniques utilize large amounts of organic solvents such as acetone and water. This presentation will focus on sustainable processing techniques for separation of PL from crude seed oils, de-oiling crude lecithin produced during conventional degumming processes and enrichment of desirable PL, i.e., phosphatidyl choline (PC) in the final product. Applications of enzyme aided processing and supercritical fluid technology in PL recovery from commodity oils, i.e., canola, and specialty oils such as wheat germ oil will be highlighted. Opportunities and challenges involved in sustainable PL production will be discussed.

**Improvements in the Extraction of Milk Phospholipids from Beta-serum Using Ultrasound Prior to Tertiary Amine Extraction.** Sergio Martinez-Monteagudo, New Mexico State University, USA; Kaavya Rathnakumar, South Dakota State University, USA

We report improvements in the extraction of milk phospholipids (MPLs) from beta-serum, a dairy byproduct, by applying ultrasound prior to a tertiary amine extraction. Three acoustic intensities (15.53 $\pm$ 1.20, 31.76 $\pm$ 2.46, or 44.56 $\pm$ 3.47 W cm-2) were applied for 4 min before the amine extraction (N,N-dimethylcyclohexylamine, CyNMe2). The extracted lipids were fractionated by solid phase-microextraction, and the recovered MPLs were quantified by HPLC-CAD. An acoustic intensity of 44.56 $\pm$ 3.47 W cm-2 followed by CyNMe2 extraction (12/1, solvent to ratio ratio) yielded 69.67 $\pm$ 3.45% of MPLs, while only 7.57 $\pm$ 0.59% were recovered without ultrasound. The fraction of MPLs was made of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%). Scanning electron images and particle size revealed significant disruption of the complex arrangement between membrane proteins and MPLs, which may help to release the MPLs into the aqueous medium. Upon further separation, MPLs can be used as ingredients for a number of applications, such as infant formula and instant powders. This study provides a road map for the use ultrasound prior to CyNMe2 extraction of MPLs from beta-serum.

**Phospholipid Recovery from Sweet Whey and Whey Protein Concentrate: Use of Electrodialysis with Bipolar Membrane Combined with a Dilution**. Mélanie Faucher, Université Laval, Canada; Véronique Perreault, Université Laval, Canada; Ozan Ciftci, University of Nebraska-Lincoln, USA; Sami Gaaloul, Lactalis Canada, Canada; Laurent Bazinet, Laval University, Canada

In the last few years, there has been an increasing interest over the dairy phospholipids (PLs) as they are a valuable source of sphingomyelin and phosphatidylserine. These two classes of PLs can have a positive impact on brain, aging, neurodegenerative diseases, etc. Sweet whey (SW) and whey protein concentrate (WPC) could be an attractive source of dairy PLs and electrodialysis with bipolar membrane (EDBM) seems promising to recover PLs, as it was already used to recover residual lipids. EDBM promotes lipoprotein complex formation following a decrease in pH and ionic strength: the complexes are found in the precipitate after centrifugation. The aim of this work was to study the impact of dilution factor after EDBM treatment on the process performances, particularly on the recovery of PLs. Hence, SW and WPC were treated by EDBM until a pH 4.5 was reached and after treatment 4 dilution conditions were tested: without dilution and with a 2X, 4X and 6X dilution. For SW and WPC, higher defatting rates were reached when a dilution was applied after EDBM, but the highest rates were not necessarily reached for the highest dilution factor, since a plateau was reached at a 4X dilution, which corresponds to a decrease in ionic strength of around 80 % and seemed to be sufficient to favor lipoprotein complex formation. For the first time, it was demonstrated that the process can be used to recover PLs. Higher PL content were found in the precipitates recovered from SW combined with a dilution after EDBM. For both SW and WPC, the main PLs found in the precipitates were phosphatidylethanolamine and phosphatidylserine. Considering the defatting rate reached, the precipitate composition, particularly in PLs and proteins, and the ecoefficiency scores the most interesting condition tested was SW combined with a 2X or 4X dilution after EDBM.

**Selective Extraction of Phospholipids and Minor Lipid Components from camelina Sativa Seed Using Ethanolmodified Supercritical Carbon Dioxide.** Ozan Ciftci, University of Nebraska-Lincoln, USA; Henok Belayneh, Terviva Bioenergy, Inc., USA

Camelina sativa seed is an underutilized oilseed that attracted interest for biofuel production. In recent years, there is a growing interest to camelina seed as an edible oil source due to its high omega-3 fatty acids and minor lipid components such as tocopherols, phytosterols, and phospholipids. Its fatty acid composition was reported before but there is little information on its phospholipid and minor lipid composition. On the other hand, its high omega-3 content and growing demand for clean food processing technologies make conventional oil extraction less attractive. Therefore, there is a need for alternative clean and sustainable methods to extract camelina seed lipids, including phospholipids.

In this study, effect of extraction methods on the bioactive minor lipids and phospholipid composition of the camelina seed oil was investigated. Camelina seed oils were extracted with ethanol-modified supercritical carbon dioxide (SC-CO2) as a green extraction method, and compared with cold press and hexane extractions. Ethanol-modified SC-CO2 extractions were carried out at varying temperatures (50 and 70 °C), pressures (35 and 45 MPa), and ethanol concentrations (0–10%, w/w). The highest total lipid yield (37.6%) was at 45 MPa/70 °C with SC-CO2 modified with 10% (w/w) ethanol. Phospholipids and phenolic content increased significantly with ethanol-modified SC-CO2 ($p < 0.05$). SC-CO2 with 10% (w/w) ethanol concentration selectively increased phosphatidylcholine content.

Ethanol-modified SC-CO2 extraction allowed modification of the lipid composition that was not possible with the conventional extraction methods. This is a promising green method for selective extraction and fractionation of camelina seed phospholipids.

**Study of Scalable Processes for Enriching Dairy Phospholipids.** Tong Wang, The University of Tennessee, USA

Dairy PLs are unique in composition and nutritional properties. There is a strong interest in recovering such polar lipids for high-value applications. However, the low content of PLs in dairy products and by-products makes it difficult to extract and concentrate. This presentation will demonstrate using solvent extraction methods to obtain high concentration of PLs in three studies. First, ethanol extraction of dairy PLs is shown to be only modestly successful if both high recovery and high concentration are desired. Secondly, fractionation of the total dairy lipids using several solvents and temperatures shows that using acetone at ambient temperature can lead to high PLs yield and product purity. Thirdly, salt precipitation of the dairy membrane fragments from dilute or low-solid by-products is evaluated, and subsequent solvent extraction from the precipitate is shown to be effective in obtaining PLs concentrate. These studies demonstrate that dairy PLs can be feasibly recovered from processing by-products using scalable methods. Development of greener technologies will lead to dairy PLs products of consistent composition and quality to enable better controlled nutritional studies and commercial applications.

## Phospholipid Poster Session

*Poster Chair Zacchary Cooper, Utah State University, USA*

**Extraction of dairy phospholipids using switchable solvents.** Kaavya Rathnakumar, South Dakota State University, USA; Sergio Martinez-Monteagudo, New Mexico State University, USA

Milk phospholipids (PLs) are recognized for their health benefits. Currently, extraction of the PLs fraction from concentrated streams results in low efficiency, and it involves subsequent solvent separation and lipid recovery. This research aims at developing a PLs extraction process from dairy byproducts using switchable solvents. The new generation of solvents are smart and can be reversibly switched between a hydrophobic and hydrophilic form by simply bubbling or removing CO2 and can be reused. This novel approach requires neither distillation nor the use of volatile, flammable or chlorinated solvents. Different dairy by-products streams were used to evaluate the feasibility of switchable solvent extraction. A tertiary anime (N, N-dimethyl cyclohexylamine, CyNMe2) was used as a switchable solvent. The recovered PLs were quantified by thin-layer chromatography and HPLC-CAD. For comparison, PLs were also extracted using the Folch method (FM) and the Mojonnier method (MM). Statistical analysis was carried out to identify the significance of different process parameters. The extraction efficiency of CyNMe2 ranged from 0.33–99%, depending on the type of byproduct. Remarkably, the CyNMe2 extracted up to 99% of the PLs directly from buttermilk. Improvements in the extraction efficiency of PLs from B-serum was done by the effect of acoustic intensity ($44.56 \pm 3.47$ W cm-2) before CyNMe2 extraction (1/12mL) ratio recovered $69.07 \pm 0.11$% of PLs, a 9.8 fold increment than the samples without ultrasound-pretreatment. The recovered fraction of PLs mainly comprised of phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%). Optimization was done with maximum desirability of 0.982 and the model was significant. The model was significant for all three responses and the coefficient of determination(R2) were 0.9825, 0.8164 and 0.7060 for 3, 10, 18 h. For CyNMe2, scanning and confocal microscopy-images, particle size, and gel electrophoresis revealed great disruption of the protein matrix, releasing the PLs into the aqueous medium.

## Processing

*Division Vice Chair: Usha Thiyam-Hollaender, University of Manitoba, Canada*

## Best Practices and Guidelines for Plant and Product Personnel—Including Public and Customer Communication

*Chairs Bryan Yeh, American Biodiesel Dba Community Fuels, USA; Stan Pala, Solex Thermal Science, Inc, Czech Republic*

**Best Practice for Oilseed Flake Rollers to Improve Lifetime and Oil Yield.** Thorsten Muenker, Walzen IRLE - SIWACO GmbH, Germany

The world population is still increasing on a yearly basis and so does the consumption oilseed per year. Within the Oil-preparation process the cracking and flaking of the oilseeds has a tremendous influence on the results in the oil yield.

Studies have shown that an optimum thickness of the flakes varies between 0.012" to 0.016" (0,30 to 0,40mm), which is reducing the milling defect. Current practice varies between plants but shown in general that the flakes have too much variation in flake thickness (from one end to the other end of the flaking roll surface). This causes oil to be passed through the process without being extracted and results in loss of profits. This could be avoided by improved rollers, roll materials and maintenance.

Consequently, improvements in roll materials and maintenance roll exchange programs have to be installed. Therefore the geometry of roundness, cylindricity and TIR of the roll has to be improved and the tolerances in between the roll nip have to be tighter to guarantee a uniform flake thickness which results in a lower milling defect.

Time to maintain the equipment is always a critical issue for the plants while the pressure of reaching the capacity is another target.

Our study focuses on the improvement of the lifetime of the roll and the higher profit for the processing plant due to better oil yield.

**Improvements in the Use of Silica Technology in Edible Oil Refining Processes.** John McNichol, PQ Corporation, USA

Justification: Silica gel has been well established as a processing adjunct in edible oil refining. As refiners have become further challenged by tight margins, silica technology can help reduce overall processing costs. Recent improvements in product and processing methodology have helped refiners achieve greater margins via reduced operating expense.

Objective: The use of silica in various refining operations including modified bleaching and water wash replacement will be shown to reduce operating expense and minimize environmental impacts.

Methods Used: Data have been developed in the laboratory, in plant trials, and empirically demonstrating the advantages of incorporating silica technology. Economic models of contrasting technologies are then created to verify the benefits.

Results: The use of silica has effectively replaced water wash in many cases not only providing an economic incentive but environmental benefits as well. Similarly, inclusion of silica gel with the bleaching step has demonstrated overall lower processing costs and shown positive environmental impact vis-a-vis reduced filter cake generation. World-class plants have realized 100's of thousands of dollars in annual savings through reduced chemical buy, feedstock losses and waste disposal costs.

Significance: The inclusion of silica technology has demonstrated economic benefits to edible oil processors in a myriad of ways including reduced chemical buy, lower utility costs, reduced environmental fess, lower utility costs and increased product yields.

**Manufacturing of Organic Oils & Meal.** Matthew Williamson, ADF Engineering, USA; T. Williamson, P.E., ADF Engineering Inc., Miamisburg, OH, USA.

U.S. growth in consumer demand for organic oils and meal has outpaced demand for traditional oilseed products, resulting in imports of organic soybeans, changes to manufacturing processes and the evaluation and implementation of a variety of new Non-GMO feedstocks. Consumer concerns over hexane use in extraction have resulted in a resurgence of Mechanical Extraction technology and a need for segregated feedstock and oil handling equipment and piping.

How do you integrate organic oils into your existing process? How can these new production demands be dealt with inexpensively? This presentation will discuss the impacts of organic oilseeds manufacturing and strategies for successful implementation.

**Water Footprint Sustainability Playbook for OILSEEDS Processing Plants.** Rakesh Patel, ADF Engineering, USA

Environmental regulations pertaining to water use and discharge have increasingly become more stringent across the United States in the past decade. Fresh water is a scarce resource with limited availability and increasing demand. Inability to manage water resources in a sustainable manner poses operational and financial risks for OILSEEDS facilities. Corporate Social image is also an important consideration for consumers and internal workforce.

This presentation will focus on a step-by-step approach tailored for OILSEEDS facilities to reduce water footprint by identifying opportunities for reducing water use through process optimization and recycling water for beneficial use within the facility. Best practices related to establishing benchmarks, developing plans, and executing water reduction initiatives will be discussed with case studies.

## Cannabis and Hemp Processing—New Trends and Applications

*Chairs Jerry King, CFS, USA; Michael Martinez, Meadowfoam, USA*

**Characterization of By-products from Commercial Cannabidiol Ethanol Extraction.** Francisco Leyva

Gutierrez, The University of Tennessee Knoxville, USA; John Munafo, University of Tennessee Knoxville, USA; Tong Wang, The University of Tennessee, USA

The chemical compositions of by-products from commercial cannabidiol (CBD) extraction were characterized and quantitated by employing gas chromatography mass spectrometry (GC–MS) and GC flame-ionization detection (GC–FID). The four major by-products included an ethanol-wax suspension (WAX), terpenoid distillate (DIST-A), tar-like residue (TAR), and red resin (RES). The composition of WAX consisted of ~28 wt % n-alkanes and ~33–38 wt % cannabidiolic acid and CBD combined. The DIST-A consisted of ~40 wt % sesquiterpenoids and ~58 wt % cannabinoids. The DIST-A terpenoid profile was compared to dried unprocessed inflorescences (HEMP) to observe changes in monoterpene content after the distillation process. The TAR was composed of ~5–9 wt % higher n-alkanes and up to 91 wt % cannabinoids, while RES consisted of up to 99 wt % cannabinoids. Several impurities including cannabidibutol and dehydroabietic acid were identified in commercial CBD samples. Compositional information of these by-products may provide manufacturers with the opportunity to optimize processing conditions.

**Extraction of Terpenoids and Cannabinoids Using Different Organic Solvents.** Tao Fei, University of Tennessee, USA; Tong Wang, The University of Tennessee, USA

Different organic solvents including diethyl ether, pentane, and ethanol were tested for the extraction of terpenoids and cannabinoids from hemp inflorescence. The effect of pretreatment such as grinding and extraction conditions such as the number of extractions, temperature, and soaking time was evaluated. The chemical profile of the extracts obtained from extraction by the different solvents under various conditions was characterized. Grinding to reduce the particle size of the inflorescence did not dramatically increase the total extraction yield from three sequential extractions, however, significantly increased the extraction yield of the terpenoids and cannabinoids of the first extraction. Among the three solvents, ethanol resulted in the highest total extraction yield (18.6 wt %) from the ground samples, while diethyl ether and pentane had better selectivity towards the terpenoids and cannabinoids. Increase the extraction temperature from 4 to 20 ºC improved the total extraction yield for all three solvents, however, further increasing the extraction temperature to 30 °C did not result in further improvement. The majority of the compounds can be extracted by the first extraction at a temperature of 20 °C. While at 4 and 30 °C, a second extraction was necessary for an equal total extraction yield.

**Phytocannabinoid-rich Extracts from Industrial Hemp Wastes by Different Extraction Techniques.** Erika Maria Vagi, Budapest University of Technology and Economics, Hungary; Margit Balázs, Bay Zoltan Nonprofit Ltd., Hungary; Attila Komoczi, Bay Zoltan Nonprofit Ltd., Hungary; Máté Mihálovits, Budapest University of Technology and Economics, Hungary; Edit Székely, Budapest University of Technology and Economics, Hungary

Hemp threshing residues originated during industrial hemp processing can be valuable sources for extraction of cannabinoids, mainly non-psychotropic compounds, such as cannabidiol (CBD). These industrial cultivars contain minimal amount of Δ9-tetrahydrocannabinol (THC) (≤0.2%) therefore their processing is more convenient without any legal burden.

In this work six hemp residues from different sources and variants were extracted with different solvents in laboratory and pilot plant scales applying different technologies. Traditional solvent extraction and high pressure processes were compared applying n-pentane, 96% ethanol, sub-, supercritical carbon-dioxide (scCO2) and scCO2 with ethanol co-solvent. The high-pressure extraction was studied between 8–45 MPa pressure and 40°C with fractionation separation at different separator pressures (4–16 MPa). Main cannabinoids and THC were quantified in the extracts by GC-MS. Extraction yields were between 2.04±0.03–4.32±0.25 % with n-pentane, 6.70±0.34–17.74±1.60 % with 96% ethanol, while scCO2 yielded 0.2–9.2 g lipophilic extract/100 g dry mass (d.m.). As the initial compositions of non-(NPC) and psychoactive cannabinoids (PC) in the residues were diverse, the extracts contained these compounds in wide ranges. Solvent extractions yielded 200.87–1415.85 mg NPCs/100 g d.m. and 15.08–64.30 mg PCs containing extracts. The enrichments of NPCs and PCs were slightly higher in the extracts obtained with scCO2 and scCO2-ethanol entrainer, as NPCs were between 227.48–1832.91 mg/100 g d.m., while PCs were 14.28–88.95 mg/ 100 g d.m. These extracts are free of solvent residues and can comply with the high-quality standards of pharmaceuticals and food ingredients. Furthermore, the optimization of high pressure extraction was carried out by investigating the effects of process parameters on the extraction yield and the enrichment of high-valued cannabinoids.

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**Preparation of Nitrogen-doped Hemp Fiber Mesoporous Carbon: Application for Removal of Naphthenic Acids from Aqueous Solution.** Paul Charpentier, Western University, Canada; Manju Gurung, Western University, Canada

High quality carbonaceous materials such as carbon nanotubes or graphene are opening the doors to new technologies in environmental remediation and alternative energy. However, LCA analysis shows these are generally energy intensive processes for their production, leading to high costs for large scale commercial employment. By using renewable biomass feedstocks such as hemp fiber can potentially produce similar high-quality materials at lower cost. This talk will describe our research work towards developing a single step process and integrating N functionalities onto the surface of the hemp carbon for the sustainable development of porous carbon materials of improved properties. We firstly investigated the efficacy of ZnCl2 as an activating agent on the surface area, pore size distribution, and pore volume of the activated carbon produced from hemp fiber, optimizing the experimental parameters. Then, nitrogen-doped mesoporous carbon materials (abbreviated as N-HFCs) were prepared by activation and carbonization of hemp fibers using N-aminoguanidine as the nitrogen source. The BET surface area, pore volume, and pore size of the hemp carbon prepared under optimal conditions were 2518 $\pm$ 19.59 m2/g, 1.546 $\pm$ 0.047 cm3/g and 24.56 $\pm$ 0.725 A$^{\circ}$, respectively, which are significantly higher than that of commercially available carbon products such as granular and porous activated carbons. Nitrogen contents of up to 12.3 w% were prepared with BET surface areas to 753.98 m2/g. The prepared N-HFCs demonstrated outstanding potential as adsorbent for removal of model naphthenic acids from contaminated aqueous solutions. Canadian tailing ponds are a well known problem arising from bitumen processing, in which naphthenic acids are produced, which are challenging to remediate using conventional approaches.

**Simple Process to Isolate Relatively Pure Cannabinoids from Marijuana and Hemp.** Albert Dijkstra, N/A, Belgium

Now that more and more states and countries allow the sale of cannabinoids for medicinal and/or recreational purposes, the need for isolation processes that are robust and economical and provide sufficiently pure products is increasing. Currently used extraction processes employing solvent like carbon dioxide, butane and alcohols have the disadvantage that they require expensive equipment because a high pressure of flammable solvents are involved. These solvents also extract many more plant constituents than just cannabinoids. Consequently, the resulting extracts are far from pure. The necessary purification steps are costly and also lead to product losses.

In this presentation, a simple process will be described in which the cannabinoids are extracted from the plant material by using triglyceride or mineral oil. Like the other solvents, these oils also extract unwanted plant constituents so the process to be discussed also comprises a further isolation of the cannabinoids. This isolation can either be effected by steam stripping under vacuum or by liquid extraction using alcohol. The steam stripping variant has the advantage that terpenes are isolated at the same time and is therefore particularly suited for THC. The alcohol route is preferred for the isolation of CBD for medicinal purposes.

The extraction of the plant material can be carried out in such a way that all cannabinoids are extracted. The isolation of the cannabinoids can also be optimized to minimize loss of cannabinoids while attaining their desired purity.

**Supercritical Extraction of Hemp Seed By-product Fractions for the Production of High Protein Content Products and High Value Extracts.** Campbell Ellison, Callaghan Innovation, New Zealand; Teresa Moreno, Callaghan Innovation, New Zealand; Kirill Lagutin, Callaghan Innovation, New Zealand; Tina Fenton, Callaghan Innovation, New Zealand; Andrew MacKenzie, Callaghan Innovation, New Zealand; Dawn Scott, Callaghan, Innovation, New Zealand; Owen Catchpole, Callaghan Innovation, New Zealand

Hemp seeds (Cannabis sativa) are very good sources of lipids rich in omega 3 and 6 fatty acids. Cold-pressing of oil from whole or dehulled hemp seeds gives press cake by-products with high-protein but low value. The hemp hulls from dehulling are similarly of low value. There is a need to recover value from these seed processing fractions. The objective of this work was to determine if further processing of each fraction by extraction and physical separation could increase protein content in the residue and/or result in high value extracts. Extraction of lipids was performed using one or more of supercritical CO2, (SCO2) subcritical propane (SP) and subcritical dimethyl ether (DME) from seed cakes, ground hulls and seed hearts. Ground hulls were also extracted with ethanol/water to recover phenolic antioxidants. Fractionation of seed meal by coarse grinding and sieving either before or after extraction was also performed. The extracted lipids and residual marcs were analyzed for fatty acids, neutral lipids, phospholipids and tocopherols. Both SCO2 and SP extracted practically all the non-polar lipids from cold-pressed hemp seed meal, giving a marc with 35.2% protein and PUFA-rich extracts. Sieve separation of the marc gave a fine fraction contained 70% protein and a coarse fraction rich in fibre and antioxidants. The DME marc from whole hearts was higher in protein (75.5% vs. 64.1%) and phytate (10.5% vs. 7.6%) and lower in moisture (5.2% vs. 9.4%) and lipid (2.6% vs. 9.0%) than the SP marc. The hemp hulls were also ground to a fine powder and firstly extracted with DME to remove lipids, and then ethanol/water mixtures to extract phenolic antioxidants including ligninamides. These technologies can be utilized to produce high-value lipid, and protein products and provide rich

sources of antioxidant polyphenols from low value hemp seed processing fractions.

## Co-Stream to Mainstream—Protein Quality and Dairy

*Chairs Lingyun Chen, University of Alberta, USA; Amar Mohanty, University of Guelph, USA*

**Attenuation of Sinapic Acid and Sinapine-derived Flavor-active Compounds Using a Factorial-based Pressurized High-temperature Processing.** Ruchira Nandasiri, University of Manitoba, Canada; Erika Zago, University of Manitoba, Belgium; N A Michael Eskin, University of Manitoba, Canada; Usha Thiyam-, Hollander, University of Manitoba; Canada

De-oiled canola sources of protein contain flavor-active phenolic compounds. Conventional canola oil processing generates a dominating bitter flavor, which limits the production of protein isolates and concentrates in food, feed, and other specialty ingredients. Recent advances have applied new technology for the isolation of canola protein for human consumption. Consequently, extraction of the bitter-favor active molecules and associated complexes will facilitate new side or co-processing streams, while elimination of bitter contributors will enhance the protein functionality. High temperature and pressure-aided processing, namely the accelerated solvent extraction (ASE) was investigated to extract the flavor-active bitter molecules. This study demonstrated that ASE impacted the extraction of major sinapates, kaempferol derivatives, and other thermogenerative compounds including thomasidioc acid (TA). The effects of temperature, solvent type, solvent concentration, and particle size, were examined on the extraction efficiency of these phenolic compounds. Extraction temperature (180oC) was the primary determinant ($p < 0.05$) for the attenuation of major sinapates including sinapine and sinapic acid. Both ethanol and methanol extractants at a concentration of 70% (v/v) significantly ($p < 0.05$) extracted the flavor-active phenolic compounds. The pressurized high temperature through optimized ASE conditions attenuates the bitter undesirable flavor and potential application to maximize the physico-chemical functionality of protein products.

**Challenges of Cold Pressing in Oilseed Processing.** Eric Stibora, Anderson International Corp, USA

Cold pressing of specialty oilseeds is growing in population due to the limitation of heat exposure to the heat sensitive organic compounds present within the oilseed. While heat can be detrimental to overall product quality, heat is an essential tool in the preparation of the material to maximize oil recovery in mechanical pressing. The fat is trapped within a cell membrane. Heat is applied in a constant moisture environment to cook the oilseed. The cooking process promotes the rupture of the cell structure that encapsulates the fat. Cooking also solidifies fluid state proteins, deactivates anti-nutritional factors, and decreases oil viscosity. After cooking is complete, the temperature of the material is further increased above the boiling point of water in order to drive off excess moisture before the pressing step. All of which, allows for a more efficient separate of the fat from the solid components of the seed.

In cold pressing, the processor is no longer able to utilize heat in the preparation of the seed or must do so within industry defined limits. The heat limitation greatly increases the difficulty of separating the oil from the material resulting in lower levels of oil recovery in the press. To compensate for the greater difficulty of oil recovery, Anderson employees a dual pressing screw press designed with high torque availability.

The Anderson Super Duo™ press has two pressing sections in a single machine. In combination with high applied torque than a traditional screw press, the Super Duo™ Expeller® can maximize oil recovery on cold pressing application in a single pass. While little to no heat is utilized upstream, the press itself will generate frictional heat. To combat the natural generation of heat in the pressing process the Super Duo™ press comes equipped with a water cooled shaft and circulating cooling oil.

**Downstream Corn Oil from Bioethanol Industry: A Versatile Green Feedstock for Advanced Material Application.** Manusri Misra, University of Guelph, Canada; Boon Peng Chang, University of Guelph, Canada; Suman Thaker, University of Guelph, Canada; Amar Mohanty, University of Guelph, USA

Vegetable oil (VO) is an abundant sustainable resource which is non-toxic and low in cost. In recent years, their applications have been expanded tremendously in the polymer industry. The unsaturated chains in VO structure allow for many possible chemical modifications and functionalizations such as epoxidization, hydroxylization, esterification, etc. With proper modification, the modified VO can be used as a lubricant, plasticizer, toughening agent, biobased resin or hydrophobic coating. In this work, a few approaches have been presented to modify VO obtained from downstream corn oil (a co-product from the bioethanol industry). Multifunctional corn oil was successfully synthesized through a ring opening process with formic acid and hydrogen peroxide. Plastic products are usually flammable due to their carbon-based rich backbone structure. Hence, flame resistant plastic products are commonly desirable for engineering applications. Halogen-free biobased flame retardant (FR) from downstream corn oil was successfully synthesized through phosphorylation process with phosphorus acid. It was found that the flame retardancy of engineering

plastics was improved after incorporation of our biobased FR. In another approach, the downstream corn oil was modified with itaconic acid and a crosslinking agent to obtain an effective toughening agent for polylactic acid (PLA) modification. It was found that the elongation of PLA enhanced remarkably (18 times) with addition of small amounts of the modified oil. Finally, a novel approach to develop a sustainable super hydrophobic coating from downstream corn oil will also be discussed.

**Proteins from Cold-pressed Canola Meal with Excellent Functionalities.** Jingqi Yang, University of Alberta, Canada; Zhigang Tian, University of Alberta, Canada; Lingyun Chen, University of Alberta, USA

Canola is one of the largest cash crops in Canada. Abundant and affordable proteins can be extracted from canola oil processing waste, the canola meal. Conventionally, the high temperature and organic solvent treatment during canola oil process have negative impact on the canola protein functionality and nutritive value. The applications of canola protein were hindered by their functional properties and dark color. Thus, cold-pressed canola meal has potential to be a source of high-quality canola protein. This study aimed to develop efficient and mild extraction methods to obtain canola proteins from cold-pressed canola meal for improved functionalities. This research reported canola protein extraction by an enzyme-assisted method from cold-pressed meals. The prepared canola proteins possessed higher solubility and emulsifying properties at pH 5 and 7 as compared to that extracted by high alkaline extraction method. In addition, the extracted canola protein had good gelling capacity, forming gels at relatively low protein concentration (12%) by heating at 90°C. As compared to commercial canola protein extracted from hot-pressed or solvent-treated canola meal, canola proteins extracted from cold-pressed meal with enzyme assisted method showed good functional properties, which have potential to be widely used in food and non-food applications.

## Emerging Processing Technologies

*Chairs Richard Clough, Texas A&M Engineering Experiment Station; Rich Barton, N Hunt Moore & Associates, USA*

**An Innovative Approach to Rapeseed/canola Processing.** Michal Kavalek, Farmet AS, Czech Republic

Rapeseed is a well-known oilseed, mainly popular for low erucic cultivars that allow the widespread use of canola oil for human food production. Today it is the world's third most commonly grown oilseed. After the ban on meat and bone meals, EU has seen a great surge in the usage of extracted rapeseed meals (RM). With a sufficient supply of RMs and an aim to relax the dependency on imported soybean meals (SMs), rapeseed meals have become widely used in the dairy cows nutrition. Today they form a common component of feed mixtures. RMs are distinguished by their high protein applicability, which makes them in many regards a better feed choice than SMs. To a lesser extent, rapeseed products are also used in the nutrition of monogastric animals (pigs and poultry). This is mainly because they are rich in fiber and digestibility-reducing substances (such as glucosinolates).

New approach to processing has been developed with a focus on eliminating the negative properties of rapeseed products. The core of this technology consists in dehulling and expeller-pressing with extrusion. While all these technologies have been around for a number of years, the novelty lies in approaching the by-product - the seed hulls. In our technological solution, these are also processed through screw-pressing, which allows a much more intensive and efficient dehulling process, which would otherwise lead to an unacceptable increase of the hull-containing waste fraction. In our innovative technology there is no 'waste fraction'. The use of extrusion serves mainly the purpose of raising oil yield, reducing the amount of antinutritional substances, and lowering the solubility of fiber. All of this increases the usability of the nutrients in rapeseed products. The technology thus yields three basic products: crude vegetable oil, high-protein cake for monogastric nutrition, and low-protein cake for ruminant nutrition.

**Canola Sprouting as Dehulling Mechanism and High-quality Meal for Food and Feed Applications.** Ahmad Alhomodi, South Dakota State University, USA; Mark Berhow, USDA ARS NCAUR, USA; William Gibbons, South Dakota State University, USA; Ewumbua Monono, North Dakota State University, USA; bishnu karki, South Dakota State University, USA

Canola is an important oilseed crop providing high level of oil (40–45%) and protein (~25%). The extraction of oil from canola seed by mechanical press or organic solvent results in large quantities of protein-rich (38–43%) meal as a co-product. Canola meal has very limited market opportunities due to the presence of high levels of fiber and antinutritional factors (phytics and glucosinolates). Some attempts have been made to mechanize dehulling canola seeds, but there is no general industrial-scale technique for dehulling Brassica species seeds. Thus, we used three-day canola seed sprouting as a dehulling mechanism and to provide nutritive meal for food and feed applications. The optimized sprouting and dehulling process resulted in 63.17% sprouts, 29.34% mix fractions (MF) (hulls, ungerminated seed, and delayed sprouts), and 8.14% mass loss during germination. Fresh sprouts and MF were dried, ground, and defatted to compare the

obtained meals and oils with their counterparts of raw seed. Defatted sprouts (DFSP) resulted in a reduction of 46.2% in crude fiber, 34.3% reduction in acid detergent fiber, and 43.4% reduction in neutral detergent fiber as compared to defatted raw seed (DFSE). DFSP provided a 10.1% higher protein content and a 5.95% increase in total amino acid content with higher essential amino acids as compared to DFSE. Total carbohydrate was lowered by 5.5%, phytic acid content was lowered by 25.9%, and the ash content was lowered by 5.47% in DFSP, whereas the total glucosinolates content was higher in DFSP (13.1 μM/g) than DFSE (8.8 μM/g). Sprouts and MF showed an oil content of 38.43% and 9.64% as compared to raw seed (34.50%). Sprout oil contained higher amounts of unsaturated fatty acids and free fatty acids, but less saturated fatty acids.

**Characterization of Rapeseeds Wettability by Capillary Rise Method.** Yancie Gagnon, Université de Technologie de Compiègne (UTC), France; Houcine Mhemdi, UTC-ESCOM, USA; Frédéric Delbecq, UTC-ESCOM, France; Elisabeth Van Hecke, UTC, France

Wettability is a property of solid-surfaces towards liquids that has become a standard performance criterion in many fields such as coatings, dispersions, and recently crude oil recovery. The wettability principles depict how a liquid spread over a solid-surface. The wettability is generally expressed as the contact angle of a liquid droplet placed on the solid-surface. The direct measurement of the contact angle is however limited to ideal (ie continuous, planar, and homogeneous) solid-surfaces. In the case of non-ideal solid-surfaces, like porous solids or powder beds, indirect methods like the Capillary Rise method have been developed. Capillary laws lead to equations connecting the rising of the liquid through the solid media and time. Such relationships include, of course, the contact angle and the physicochemical characteristics of the solid (ie. porosity) and the liquid (ie. bulk density, viscosity, and surface tension). Washburn's law is the basic capillary rise model. It considers the media as a unique irregular capillary tube whose geometrical characteristics (mean pore radius, tortuosity) can be determined beforehand with a liquid that is assumed to perfectly wet the solid.

Since the wettability of oilseeds has never been reported in the literature, this work is devoted to study the wettability of rapeseeds materials especially. For this purpose, the capillary rise method was operated with different liquids (alkanes, water, green solvents, and rapeseed oil). Mass versus time curves were standardized in order to compare the behavior of the liquids. Atypical results were obtained, especially with alkanes, which were thought to be perfect wetting liquids. The classical Washburn law has been modified to consider possible swelling of the media and to adjust to the data. Results provide a new understanding of the behavior of the complex ternary oil-seeds-solvent systems as encountered in the oil extraction process.

**Effect of Different Dilution Solutions in Palm Oil Mill Towards Prevention of 3-MCPD Formation in Refined Bleached Deodorised Palm Oil (RBDPO).** Syed Mohd Hadi Syed Hilmi, Sime Darby Plantation Research Sdn. Bhd., Malaysia; Norliza Saparin, Sime Darby Plantation Research, Malaysia; Rahmat Ngteni, Sime Darby Plantation Research Sdn., Bhd., Malaysia; Yosri Mohd Siran, Sime Darby Plantation Research Sdn. Bhd., Malaysia

This paper examines the effect of dilution water available in the palm oil mill and how they affect manifestation of 3-MCPD later in the refinery stage. Dilution of crude palm oil in mills is done to enhance fluidity of the oil after pressing and facilitate the separation process in clarification stage. A set of different dilution solutions - hot water, steriliser condensate (SC) and empty fruit bunch (EFB) liquor that are available inside the mill were used to dilute the undiluted crude oil (UDCO) at 30% rate, except for mixture of steriliser condensate and EFB liquor which was at 26% plus 4% respectively. The study shows that lowest and highest amount of 3-MCPD observed was when UDCO diluted with hot water and combination of steriliser condensate and EFB liquor at 3.85mg/kg and 6.85mg/kg respectively. This is to show that hot water is the better solution to be used for dilution while giving the lowest formation of 3-MCPD and better oxidative stability to RBDPO. The difference between the dilution solutions is the chloride content they carry. SC and EFB liquor have higher total chloride content compared to water. The study also found a strong correlation between total chloride and 3-MCPD with its R2 of 0.9035. Through this relationship, it can be used to predict 3-MCPD prior to actual lengthy refining process from the crude palm oil extracted. By fixing the problem earlier in the supply chain, it could provide greater room for improvement down the processing line.

**Evaluating the Tribo-charging Behavior of Oat Bran and Endosperm Particles During Dry Electrostatic Separation Approach for Functional Protein Production.** Solmaz Tabtabaei, Howard University, USA; Dinara Konakbayeva, Howard University, USA

A novel tribo-electrostatic separation approach has been developed for milled oat groats to produce functional oat protein concentrates. Conventional protein separation processes are focused on isolating proteins through wet fractionation methods that involve the use of concentrated acids/alkali while using great amounts of water as well as energy for dehydration. Our newly developed tribo-electrostatic separation approach is a dry and waste-free replacement of traditional protein fractionation methods. We have developed a vertical tribo-electric separator consisting of fluidized bed, PTFE tribo-charger tube and a separation chamber

equipped with two oppositely charged electrodes. This approach started by suspending oat particles in air, followed by their tribo-charging in the PTFE tube through particle-particle and particle-wall contacts. The charged particles were then entered the separation chamber where the positively charged protein particles were separated from starch-rich particles, resulting in the production of functional oat protein concentrates. The study was first focused on assessing the influence of air flow rates and electric field strength on oat protein enrichment. At optimized laminar air flow rate and high plate voltage (±12kV), the protein content of starting oat flour was increased from ~10 to 18%, equivalent to protein separation efficiency of ~50%. The chargeability of oat bran and endosperm particles as a function of particle size and protein content at laminar and turbulent air flow rates were investigated. While no specific relationship was found between specific charge (nC/g) of oat particles and their mean diameters, a linear relationship was observed between the charge density (nC/m2) and protein content of the bran-rich and endosperm-rich oat particles. The charge density was linearly increased by increasing the protein content of oat particles regardless of their particle size. Understanding the charging behavior of milled cereal particles will help us in improving the electrostatic separation design while enhancing the level of protein enrichment.

**What Is Climate Change All About and How Can We Save Energy and Reduce Emissions Anyway?** Alan Paine, Independent, United Kingdom

As global warming continues to climb its way up the international agenda this talk will look at the issues behind climate change and what the vegetable oil and allied industries can do about it. We have already seen a rising interest in responsible sourcing such as sustainable palm and soybean oil and it seems likely that climate change friendliness will become an increasing part of that.

But even if we weren't worried about increasing carbon dioxide and other emissions there are still energy saving measures that could be introduced in many refineries with a quick return on investment. We will look at some of the options which could lead to long term cost savings and be worth doing whether a global disaster is around the corner or not.

## Food Safety and Security—Contaminants, Sustainability, Environment

*Chairs Nurhan Dunford, Oklahoma State University, USA; Veronique Barthet, Canadian Grain Commission, Canada*

**Can Blockchain Technology Facilitate an Effective End-to-end Food Traceability System? The Role of Trust.** Brian Adam, USDA Economic Research Service, USA

A robust whole chain (end to end) traceability system can limit consumers' exposure to potentially hazardous food contamination, improve supply chain management, and add value to consumer products. Fragmented supply chains, however, present special challenges, since these benefits depend on members of a supply chain sharing detailed information about their operations. One barrier to adoption of traceability systems is lack of trust to share these data with powerful members of the supply chain.

Proponents of blockchain technology (BT) say it has the potential to enable decentralization of trust by disintermediating institutions. Trust is decentralized when no one individual or organization can use shared data opportunistically. However, some organizations (service providers) are offering blockchain-based solutions for food traceability. This apparent contradiction motivated us to investigate whether BT can overcome barriers to adoption of traceability systems. Our findings suggest that BT applications in food traceability do not completely decentralize trust, and thus do not resolve all the important barriers to adoption. This presentation will briefly describe blockchain technology and its features, contrasting its use in cryptocurrencies and its use in food traceability systems.

**Can Blockchain Technology Facilitate an Effective End-to-end Food Traceability System? The Role of Trust.** Blayne Mayfield, Oklahoma State University, USA

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**Food Safety: Focused Facility & Equipment Hygienic Design Strategies.** Dennis McCullough, Process Plus LLC, USA

Food Manufacturing facilities are required to review their production system to determine if they have any mitigation strategies to protect food against intentional adulteration or complete their own vulnerability assessment. Once that is completed, manufacturers would need to identify actionable process steps, which are points, steps, or procedures in a food process that will require focused efforts to reduce any such risks. Facilities are also required to complete a written food defense plan. Once in place, the Food Safety Modernization Act (FSMA) would establish measures that a food facility would be required to implement to protect against the intentional adulteration of food. A key deliverable in achieving this goal is designing facilities and equipment to proper hygienic design levels.

The purpose of this research is to define how to avoid intentional adulteration to our manufactured products. Having a "Kill" step to the manufacturing process of oil processing operations is not enough in the avoidance of microbiological contamination. Beyond having hygienic designed equipment with proper cleaning and sanitization processes for low water activity products, one must understand facility design, material flows, and personnel transfers within the manufacturing environment. Before we jump to the conclusion of selecting the 'best' designs, we must provide the right technical solution(s), which is based on understanding current designs.

**Food Traceability Proposed Rule of the Food Safety Modernization Act: An Overview.** Ravi Jadeja, Oklahoma State University, USA

This presentation discusses the U.S. Food and Drug Administration's (FDA) proposed food traceability rule of the Food Safety Modernization Act (FSMA). The rule proposes additional traceability recordkeeping requirements beyond what is required by the existing regulations for persons that manufacture, process, pack or hold foods that the FDA has designated for inclusion on the food traceability list (FTL). Once finalized, the rule would require entities to establish and maintain records containing critical traceability information throughout the supply chain for certain high-risk foods. The traceability rule is intended to help FDA rapidly identify recipients of potentially adulterated or misbranded foods to prevent foodborne illness outbreaks. The presentation will cover topics such as foods included in FTL, critical tracking events, traceability program record requirements, proposed exemptions, and modified requirements, compliance dates, and potential ways to meet the proposed traceability rule requirements.

**Low 3-MCPD Ester RBDPO by Water Washing: Study of Physicochemical Properties, Evaluation of Quality and Storage Stability.** Norliza Saparin, Sime Darby Plantation Research, Malaysia

Low 3-MCPD Ester Refined, Bleached and Deodorized Palm Oil (RBDPO) was achieved by Crude Palm Oil (CPO) water washing. Physicochemical properties such as iodine value (IV), deterioration of bleachability index (DOBI), impurities, oxidative and hydrolytic parameters were measured to investigate the effect of water washing on the quality of CPO. The study was done for 120 days with two treatments, (1) CPO was store and washing using distilled water and compared with (2) washed CPO prior to store. The storage condition is controlled at 25°C. CPO quality showed washing process able to reduce impurities, iron (Fe), Copper (Cu) and Phosphorus whereas at the same time reduced total chloride which subsequently reduced the 3-MCPD Ester in RBDPO. CPO was subjected to physical refining process using lab scale set-up with 1% activated bleaching earth and deodorized at 260°C. RBDPO quality in terms of free fatty acid (FFA), anisidine value (AnV), total oxidation (TOTOX) and induction period, are comparable with untreated sample.

**MOAH in Infant Formula: Current On-goings in the EU.** Jan Kuhlmann, SGS Germany GmbH, Germany

Mineral Oil Hydrocarbons (MOH), consisting from a saturated fraction (MOSH) and an aromatic fraction (MOAH) do play an important role in our engineered world. Consequently, they can occur as food contaminants. Knowledge about migration of MOH in foods and bioaccumulation in humans has been reported since years. In the beginning, the public focus was set mainly on chocolate. Later, various contamination pathways were identified and grains & cereals as well as oils & fats and various other foods were considered to frequently be contaminated by mineral oil fractions. This raised some health concerns as especially MOAH are suspected to include genotoxic aromatic components.

After identification of MOH as possible food contaminants, NGOs reported several times on MOSH/MOAH in foods and raised public attention. Responding on this, EFSA started collection of data on the occurrence of MOH in foods. In parallel, German authorities were focusing on regulations on the migration of mineral oils from food packaging materials into foods. The issue became a hot topic when in 2019 foodwatch reported on "suspected cancerogenic mineral oil residues" in infant formula. As infants are the most vulnerable consumers, the European authorities noticed a new gap in terms of consumer protection. Unfortunately, analysis of mineral oil fractions in foods is challenging and the methods applied so far do show a lack of sensitivity and insufficient repeatability.

In terms of MOAH in infant formula, this presentation summarizes the status quo with special focus on the analytical methodology and activities of the European Authorities.

**Removal of Cyanogenic Glycosides from Whole Flaxseed Using Bacteria Fermentation.** Chao Huang, University of Saskatchewan, Canada; Sarah Purdy, Univ of Saskatchewan, Canada; Timothy Tse, University of Saskatchewan, Canada; Venkatesh Meda, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada

Flaxseed is a functional food that contains α-linolenic acid, carbohydrate, lignan, dietary fibre, vitamins, and minerals. Despite the potential use of flaxseed for health applications, flaxseed also contains some small amounts of cyanogenic glycosides (CG), including linamarin, linustatin, lotaustralin and neolinustatin. In the presence of the enzyme beta-glucosidase, cyanogenic glycosides are decomposed to cyanohydrins by the enzyme hydroxynitrile lyase to release hydrogen cyanide (HCN). This latter compound is toxic to mammalian respiration, the nervous system, and endocrine systems. In this report, a simple method is described for decreasing CG in whole flaxseed. Whole flaxseed samples were fermented with lactobacillus cultures for 48 hours in a 30°C water bath. The concentration of total HCN, linustation and neolinustin in whole flaxseed was measured using 1H-NMR before and after fermentation. Total cyanogenic glycosides in the whole flaxseed was reduced from 237mg/kg to 10 mg/kg. Both linustatin and neolinustn were reduced to less 0.05%. Whole flaxseed treated with this fermentation method is depleted in CG and may be more widely applied as a baking ingredient and in animal feed.

## General Processing

*Chairs Rich Barton, N Hunt Moore & Associates, USA; Richard Clough, Texas A&M Engineering Experiment Station*

**Large-scale Industrial Trial on Canola Using 2-methyloxolane as Extraction Solvent - A Clean Alternative to Hexane.** Mickaël Bartier, EcoXtract (Pennakem Europa), France

EcoXtract® is a new bio-based solvent derived from hemicellulose proposed by Pennakem. It is composed of a pure molecule, the 2-Methyloxolane or 2-MeOx which naturally occurs in edible plants.

Since 2012, a PhD work and several scientific papers have identified the 2-Methyloxolane as a very effective, non-toxic and cost competitive substitute to hexane for lipid extraction and protein defatting.

In 2020, to support the industrial scale-up, Pennakem carried out a trial on 45 MT of rapeseed press cake on a 10 MTPD continuous process including immersion extraction, oil and meal desolventization and solvent recovery. This presentation will detail the results of this run which allowed to produce well defatted and desolventized meal with continuously recycled solvent recovered by simple decantation (a dried meal containing less than 0,7% of residual oil and less than 15 ppm of residual solvent). The oil and meal produced using the 2-Methyloxolane were comparable in quality and quantity with hexane extraction.

The trial also provided data to feed a Chemcad process simulator which allows to estimate the utilities consumptions.

The Chemcad model was used further to deliver large scale comparative OPEX simulation between hexane and 2-Methyloxolane.

These studies allow to conclude that the solvent can be used in an existing hexane-based plant with limited modifications and with similar OPEX.

**Method for Extracting Oil from Fines in Oilseed Plants.** Richard Ozer, Crown Iron Works Co, USA

Fines in Solvent Extraction Plants tend to hinder Percolation and can hurt overall recovery of oil. In Distillation, the fines build up in tanks or on Heat Transfer surfaces eventually plugging tubes. To prevent fines from entering the plant, the fines are often aspirated or screened away from the bulk of the material and leave the plant with the hulls.

Immersion Extraction is a technique that was developed to work with finer products that sink in the solvent. Immersion Extraction also offers the opportunity to process other products that break down into fines in Percolation Extractors.

This paper will discuss the possibility of using Immersion Extraction as a means to process the oilseed "fines" as well as other non traditional products such as Alternate Oilseeds, Nutraceuticals, or Cannabis

**Simulate Plant-processing Operations, Run Product Tests, & Explore New Technologies to Save You Time and Money.** Alexander Danelich, Crown Iron Works, USA

This presentation discusses Crown's Global Innovation Center capabilities and how this facility provides customers a controlled and confidential environment with capabilities to test and develop new processes, products, or new modes of operation. This facility, along with Crown's global expertise enables customers to grow and scale-up in an efficient, continuous manner, while mitigating risks. This facility enables Crown to introduce "new to the world" processes (e.g., canola/rapeseed dehulling, rapeseed/canola protein concentrates, etc.). In addition, this world-class facility plays well in the plant-based proteins space, where customers are able to bring different varieties of soybeans, for example, to produce demonstration quantities of white flakes or SPC, that can be used for feeding trials and other testing purposes to select the best products to bring to the market. The facility is operated with a PLC and has data trending and data storage capabilities. Analytical lab

provides the means to monitor product quality and make timely process adjustments.

## New and Emerging Technology

*Chairs Jonathon Speed, Keit Spectrometers, United Kingdom; Richard Ozer, Crown Iron Works Co, USA*

**Enzymatic Degumming Process Controlled by Near-infrared Spectroscopy.** Per Nielsen, Novozymes AS, Denmark; Jakob Larsen, Copenhagen University, USA; Soeren Engelsen, Copenhagen University, USA; Klavs Sorensen, Copenhagen University, USA

Near-Infrared Spectroscopy (NIRS) instruments for process control in vegetable oil refining is already widely used. It can for example be used as in-line process control for measuring the FFA in oil, water content in meal, or composition of soap-stock. We have worked with NIRS in the laboratory to investigate the possibility of using it for process monitoring of phospholipid composition during the phospholipase catalyzed reaction in the enzymatic degumming application. First, the setup was with the instrument beside the reaction vessel and sampling during the reaction. Samples were analyzed by 31P-NMR and matched with the spectra from the instrument. It was shown, that it is possible to produce a calibration curve for the phospholipids PC, PE and PI which can then be used in future experiments for following the enzyme reaction. Next, we mounted the NIRS instrument for continuous analysis in the enzyme reactor, mimicking an in-line process control. The goal was to follow the reaction by analysis of the FFA content during the reaction. Calibration curve was established, and we have now shown how NIRS can be used as an in-line tool for process control in the enzymatic degumming reaction.

In the presentation we will share experience and data from working with the NIRS in enzymatic degumming. We will discuss the perspectives in the process control and how it can be utilized to optimize the cost efficiency of the process.

**Extraction Optimization and Scale-up of Novel Pennycress (Thlaspi Arvense) Protein Utilizing a Structure-function Approach.** Rachel Mitacek, University of Minnesota, USA; Baraem Pam Ismail, University of Minnesota, USA

Justification: Global demand for plant protein ingredients is rapidly increasing. Pennycress, a sustainable, short-season cover crop with vast environmental benefits produces a protein proficient oilseed (25–35% protein), exhibiting promise as a novel protein source. To develop a commercially-viable ingredient, effective protein extraction methods that preserve structural and functional attributes need to be optimized and investigated.

Objective: The objective of this research is to optimize protein extraction methods that maximize purity and yield and characterize the influence of extraction method and scale up on structural and functional attributes.

Methods: Oil was physically expelled from seeds. The extrudate was milled, and residual oil was removed using hexane. Protein from defatted meal was extracted by either salt solubilization coupled with ultrafiltration or alkaline solubilization and isoelectric precipitation. Optimization of each method involved various conditions: time, number, and pH of solubilizations. Optimized protein isolates from each method were subjected to structural characterization including surface hydrophobicity, protein profiling, surface charge and denaturation, using native and commercial soy protein isolate (nSPI and cSPI) as references. Functional analyses included solubility, water holding capacity, gelation, and emulsification capacity. Optimized salt extraction was scaled-up in a pilot plant and structural and functional characteristics were analyzed and compared to bench-scale protein extraction.

Results: Alkaline extracted protein was more denatured and polymerized, with higher surface hydrophobicity, resulting in lower overall functionality compared to salt extracted. Scaled-up salt extraction showed some profile differences, higher surface hydrophobicity and denaturation compared to bench-scale protein. However, scaled-up extraction had 20% higher gel strength and 40% higher emulsification capacity compared to bench-scale protein. Pennycress protein was shown to be commercially scalable with enhanced functionality favorable for food applications.

Significance: This research will expedite the commercialization of novel pennycress protein for human consumption, increasing crop value, creating incentive for farmers to plant for a positive environmental impact.

**Industry 4.0 and Advanced Process Control in Edible Oil Refining.** Richard Salliss, Keit Spectrometers, United Kingdom

From its initial development in the oil and gas industry, Advanced Process Control has become widely adopted in many segments of the process industries and has demonstrated benefits in terms of yield, throughput and energy saving many times over. Industry 4.0 has heralded the wide availability of computing and networking power along with improvements in sensor technology such as spectrometers. This allows these APC and optimisation techniques to be deployed in many new and novel industries, with the same goal of improved efficiency and profitability. Edible oil refining is an ideal candidate for such deployment.

Here we give a brief overview of the concepts of APC, introduce the underlying principles as well as discuss the potential benefits when applied to edible oil

processing. We will discuss some potential implementation routes in edible oils, and discuss strategies to make full use of APC and engage in the roll out of Industry 4.0.

**Soybean Extraction Using 2-methyloxolane as Alternative Bio-solvent - Comparative Analysis of Oil Quality & Proteins Profile with Hexane Extraction.** Ombéline Claux, Avignon University, France; Vincent Rapinel, EcoXtract (Pennakem Europa), France

Edible oils are currently largely obtained by solvent extraction using hexane that remains the state-of-the-art reference for oilseed solvent extraction such as soybean. At lab scale, we evaluated how to substitute hexane by a bio-based and safe solvent, 2-methyloxolane (2-MeOx). Analyses were focused on extraction yields, composition, and quality of the extraction products. As this study was also made to evaluate the feasibility to substitute hexane in industrial conditions, we also carried out a kinetic study and multi-stage cross-current extractions.

Experimentally, 2-MeOx gave higher crude extraction yields compared to hexane with 23.5% vs 18.8%, respectively. Also, a complete analysis of crude oils compositions revealed that main constituents were the same. Yield differences were attributed to the co-extraction of more polar additional compounds such as phospholipids and isoflavones. Crude oils extracted with 2-MeOx contained significant amounts of isoflavones (around 2000 mg GE/kg fat), while only traces were found in the hexane-extracted oil. These additional compounds were probably responsible for the enhanced antioxidant activity and oxidative stability exhibited in crude oils.

Regarding the defatted meals, no significant difference was observed in terms of protein, amino-acid contents, and anti-nutritional factors. The PDI of hexane and 2-MeOx samples were similar ($70.7 \pm 0.4$, $67.9 \pm 1.2$ respectively). The kinetic study and multi-stage cross-current extractions showed that hexane and 2-MeOx gave similar results.

**SPC and a Modified Process for Canola and Rapeseed Protein Concentrates.** Richard Ozer, Crown Iron Works Co, USA; Alexander Danelich, Crown Iron Works, USA

Most of the worlds Soy Protein Concentrate is manufactured via the Hydrous Ethanol Process developed over 50 years ago. Originally for Food, world production has more than doubled since 2004 to include the Aqua and Feed marketplace as well. Future growth is fueled by the demand for sustainable meat analogs to replace cattle, swine, & poultry.

Due to special issues related to their processing, Canola & Rapeseed are a currently untapped sources of high value vegetable protein. Recent improvements to the Hydrous Ethanol Process have made large scale processing of Rapeseed and Canola possible. This paper will focus on the hydrous ethanol process and modifications required for processing Canola and Rapeseed.

**Sustainable Chemical-resistant Nanofiltration Technology for Vegetable Oil Refining.** Mohammad Hossein Davood Abadi Farahani, Seppure Ptd Ltd, Singapore

Chemical separations are imperative for the vegetable oil, cannabis oil, and essential oil industries. Solvent recovery and separation of oil compounds using thermal evaporation processes consume a massive amount of energy. Chemical-resistant nanofiltration membrane is an energy-efficient and sustainable separation technique that can disrupt solvent recovery and free fatty acid (FFA) removal in the vegetable oil industry. Separating and purifying chemical mixtures without heat and at low temperatures without a phase change would significantly reduce energy consumption and $CO_{2e}$ emission globally. SEPPURE has developed a patented sustainable chemical-resistant nanofiltration technology that dramatically reduces energy consumption and CO2e emission for solvent recovery by enabling the most effective chemical separation at a molecular level, without the traditional use of heat. SEPPURE's technology can be used for solvent recovery and FFA removal processes for different oils, including palm oil, canola oil, soybean oil, etc. SEPPURE's GreenMem Series can recover solvents (acetone or hexane) from oil miscella (8–25**%** oil in the solvent) at >99**%** purity at a mild operating condition (30–65 °C, 15–30 bar), which results in up to 90**%** lower energy consumption and $CO_{2e}$ emission as well as 30–50**%** lower operating costs compared to the conventional distillation process. GreenMem Series membranes show a high pure acetone/hexane flux of 30–40 $L/m^2h$ as well as a high rejection towards oil molecules >95**%**. Moreover, 99**%** of FFA can be removed from a solvent/FFA mixture using two passes of membrane filtration, which can be implemented in a unique membrane system to separate oil/FFA/solvent from each other in two steps. GreenMem system can be used in both continuous and batch processes. Its modular concept makes it easy to be scaled-up based on production capacity. It is envisioned the chemical-resistant nanofiltration technology provides both positive economic and environmental impacts on the vegetable oil industry.

## Processing Basics—Current and Future Scope

*Chair Darren Litle, Arisdyne Systems Inc, USA*

**An Expert's Insight in the Separation Technology: Measuring Quality and Efficiency in the Refining of Vegetable Oils.** Patrick Schuermann, GEA Westfalia Separator Group GmbH, Germany

Centrifugal separation technology is a well proven and established technology in vegetable oil processing. Suppliers provide a wide range of different machine types with several features to adjust the machine according to actual process conditions.
There need to be always a perfect balance between quality (as good as necessary) and yield (as much as possible).
A good knowledge about the function of the above-mentioned features is essential to operate a centrifuge always with best possible results regarding oil quality, yield and chemical consumption.
Beside the above mentioned, energy consumption, process control, data and analysis and efficiency becomes more and more important and can be influenced with actual features as well.
Latest developments are features to get more process control and more and additional data from your centrifuge. Next to a higher degree of automation Online-Monitoring Systems, Cloud Monitoring & Trending and Remote Access are creating a more ‘‘intelligent’’ centrifuge.

**Benefits of Diatomaceous Earth Filter Aids Use in Bleaching Filter Operations.** Niels Mastrup, US Silica, USA
The use of diatomaceous earth is widely accepted within the edible oil market for pretreatment of freshly crushed oils seeds, winterization and polishing.
A commonly missed opportunity is the use of a first precoat of diatomaceous earth and a small amount of body feed addition to the bleaching filters. Benefits include improved flow rates, extended cycles, improved service life of filter screens, reduction of unplanned downtime due to screen fouling, reduction/elimination of clay fines to the deodorizer, plugging minor leaks. This presentation will explore the benefits of using diatomaceous earth for precoat and body feed for bleaching operations.

**Bleaching, Optimization and Key Considerations for the Adsorptive Bleaching Process.** Jorge Bello, EP Engineered Clays, Mexico
In today’s world, refineries are faced with the challenge of processing multiple types of oil with a wide range of quality. Bleaching cost optimization is one of the key factors to maintain a competitive position in the market. This presentation focuses in providing a general guideline about the parameters that have a great impact on the bleaching cost, as well as the steps for its optimization. Adsorptive bleaching involves the chemical activity of the surface used. There are different commercial products with different surface and activity which influences the bleaching efficiency, the process parameters are important to guide the adsorption and the catalytic reaction in the proper direction to reduce adsorbent dosage, oil losses and waste generation.

**Development, Implementation and Future Frontiers for Enzyme Solutions Improving Process Economics and Product Qualities Within Oils and Fats Processing – an Overview.** Hans Christian Holm, Novozymes, Denmark; Per Nielsen, Novozymes AS, Denmark; Anders Madsen, Novozymes, Denmark
30 years and counting enzymes have helped oils and fats processors increase process yield, improve product qualities and improve overall sustainability. With more than 200 oils and fats plants spread all over the World having adopted enzyme solutions; now is a good time to look at the status for current enzyme solutions and take a look forward what will come and what might come.
The presentation will offer status and future perspectives for a wide range of enzyme solutions, from specialty fats, oil extraction, oil recovery, oil refining, fat modification, biodiesel, pretreating oil for HVO, oleochemicals and more.

**Utilization of Controlled Flow Cavitation to Enhance Lipid Processing.** Darren Litle, Arisdyne Systems Inc, USA; Oleg Kozyuk, Arisdyne Systems, Inc., USA; Peter Reimers, Arisdyne Systems, Inc., USA
Crude vegetable oils contain phophatides or so-called gums that must be removed during the first step in the refining process for two main reasons: they are responsible for high refining losses due to their emulsifying properties; and they decompose, darkening the oil due to their thermal instability.
Oils that are rich in phosphatides, especially in non-hydratable phosphatides (NHP) such as soybean and canola oil, are refined by degumming with water and phosphoric or citric acid followed by neutralization with alkali and separation with a centrifuge. Each refining step results in a loss of oil yield proportional to the amount of impurities, such as phosphatides and free fatty acids present, especially due to the formation of emulsions.
Controlled Flow Cavitation has a positive impact on removing phosphatides and allows a reduction in acid consumption, which results in a reduction in alkali and, consequently a reduction in yield loss.

## Processing Division Student Eposter Pitch Competition

*Judges Alan Paine, Independent, United Kingdom; Orayne Mullings, Desmet Ballestra, USA; Hari Kiran Kotapati, USDA-ARS*

**Air frying as a pre-treatment strategy for the recovery of lipophilic sinapates from lower grade yellow mustard oil.** Olamide Fadairo, University of Manitoba, Canada; N A Michael Eskin, University of Manitoba, Canada; Usha Thiyam-Hollander, University of Manitoba,

Canada; Ruchira Nandasiri, University of Manitoba, Canada

The roasting of mustard seeds prior to oil extraction is a well-documented unit operation essential for the production of canolol and other lipophilic sinapates. This research investigated the effectiveness of air frying as a pre-heat treatment strategy for enhancing the recovery of lipophilic sinapates from Lower Grade Yellow Mustard seeds (LGYMS). Air frying of Lower Grade Yellow Mustard seeds (LGYMS) was conducted at temperature-time combinations of 160, 170 and 180 oC for 5, 10, 15 and 20 minutes, respectively. This was followed by removal of the oil. Extraction of lipophilic sinapates from the oil was achieved using an equal volume of hexane and 70% (v/v) aqueous methanol. Phenolic extracts from the pretreatment and extraction technology were quantified with HPLC-DAD. The results showed a time- temperature dependency for the recovery of major oil- soluble sinapates from lower grade yellow mustard seeds. The optimum air frying condition for the maximum yield of canolol and other major oil-soluble sinapates (RT-6.25, RT-11.50, RT-14.95 and RT-16.24) was 180 oC for 15 minutes. This information would benefit the mustard industry.

**Electrostatic conditioning of canola oil.** Li Zhou, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada; Venkatesh Meda, University of Saskatchewan, Canada

In machine shops electrostatic ''oil cleaners'' are used to remove particulate material (wear particles, soot, varnish and dust) from non-polar working fluids and low dielectric constant industrial oils. Edible oils are also non-polar fluids with low dielectric constants and typical contaminants (e.g., phospholipid, free fatty acid, peroxides, seed particles) in unrefined oil are polar particles. In this study, pilot scale tests (15 liters) were conducted on unrefined canola oil using a commercial electrostatic industrial ''oil cleaner'' (typically used for cleaning oil in a machine shop). Unrefined canola oil was passed through the ''oil cleaner'' and a rapid, single pass, reduction of free fatty acids (64.4%), b-carotene (11.8%), and peroxides (33.7%) was observed. After canola oil was repeatedly circulated through the electrostatic field for 30 cycles 74.5% of phospholipid present was removed. Canola oil quality analysis indicated that the electrostatic field did not alter canola triglyceride composition. Finally, mass balance of input crude oil and output refined oil indicated negligible neutral oil (0.37%) losses during refining.

**Formation and Physical Stability of Hazelnut Oil Nanoemulsions.** Selvi Secil Sahin, Ankara University, Turkey; Ayse Nur Akpınar, Ankara Unıversity, Turkey; Aziz Tekin, Ankara Unıversity, Turkey; Cansu Ekin Gumus, Ankara Unıversity, Turkey

Hazelnut oil, a considerable part of international industry, has not been adapted to nanoemulsion technologies, yet. The main purpose of this study is to produce nanoemulsions of hazelnut oil, a product that demonstrates cardiovascular benefits in human diet, to improve its bioavaliablity and stability. Emulsions were prepared with 2% emulsifier (Tween 80 or whey protein isolate) and 10% hazelnut oil using microfluidizer at 4–14 kpsi pressure. The zeta potential and droplet size of the samples were measured to investigate the effect of environmental factors after exposure to various conditions, such as pH (2 to 8), temperature (30 to 90°C), salt concentration (0 to 500mM NaCl), for 24 hours. Moreover, the changes during in vitro digestion and free fatty acid release were investigated. Droplet size decreased steadily with increasing pressure and number of passes (Figure 1). The use of 2% emulsifier ensured the most ideal size for both whey protein isolate (WPI) and Tween 80. Even though the particle diameter of the WPI emulsions increased at pH 5 appreciably and nanoemulsion became unstable through gelling, Tween 80 emulsions remained fairly stable (Figure 2). Especially at temperatures above 70°C in presence of 150 mM NaCl, droplet diameter increased for WPI emulsions. Nevertheless, there were no such changes for Tween 80 emulsions. During in vitro digestion, droplet size increased at stomach phase in both type of emulsions. As a result, it is determined that WPI emulsifier provides a weaker physical stability to hazelnut oil nanoemulsions than Tween 80, yet has similar strength in free fatty acid release.

**Implementation of chemical refining for the mitigation of 2-MCPDE, 3-MCPDE, and glycidyl esters: Promising pilot plant results.** Sergio Oey, Wageningen University, Netherlands; Ine van der Fels-Klerx, Ine van der Fels-Klerx, special professor, Wageningen University, Netherlands; Vincenzo Fogliano, Wageningen University, Netherlands; Stefan P.J. van Leeuwen, Wageningen University, Netherlands

Esters of 3-monochloro-1,2-propanediol (3-MCPDE) and 2-monochloro-1,3-propanediol (2-MCPDE), and glycidol (GE) are processing contaminants that can be found in refined fats and (vegetable) oils. Recently, the European Commission has implemented new maximum limits for GE, and the sum of 3-MCPDE and 3-MCPD in vegetable oils and fats. This fuels the growing urge and necessity of oil refineries for a refining method capable to minimize both the 3-MCPDE and GE concentration in their final product.

Physical refining has become the standard refining method for the majority of vegetable oils. However, physical refining often lacks the potential for the mitigation of 2-, and 3-MCPDE. Therefore, experiments were held to explore the potentials of chemical refining, providing a viable mitigation strategy ready for adoption by the industry. Experiments were performed in our pilot

plant which can hold a 100 kg of oil (Fig. 1). The pilot plant provides a good simulation of the full-scale refining process while accommodating a variety of experimental conditions such as the amount of bleaching earth, temperature, and vacuum pressure. The experiments also explored the intrinsic effects of neutralization and the effect of water washing prior to the refining process. While in operation, sample collection from the pilot plant can be done at any given moment. Quantification of 2-, 3-MCPDE, and GE were performed with an in-house validated method based on the AOCS Cd 29a-13 official method.

Compared to a single physical refining control, the chemical refining method achieved a final concentration of respectively 0.78 **(–**52%), 0.42 **(–**49%), and 0.99 **(–**73%) mg/kg for 3-MCPDE, 2-MCPDE, and GE in organic palm oil. These experiments have shown that chemical refining still has a great potential, especially for the simultaneous mitigation of 2-, 3-MCPDE, and GE.

## Processing to Enhance Bioactive Ingredients for Health-Promoting Foods

*Chairs Marc Pignitter, University of Vienna, Austria; Karin Schwarz, Kiel University, Institute of Human Nutrition and Food Science – Division of Food Technology, Germany; Karin Schwarz, Kiel University, Institute of Human Nutrition and Food Science – Division of Food Technology, Germany*

**Effect of Extrusion Processing and Coating of Extrudates with MCT Oil on the Incorporation and Oxidative Stability of Polyunsaturated Lipids in Corn Extrudates.** Jonas Amft, Kiel University, Institute of Human Nutrition and Food Science – Division of Food Technology, Germany

Extrusion cooking is a key technology of industrial food production enabling the incorporation of polyunsaturated fatty acids (PUFA) in a starch-protein based matrix. However, PUFAs in extrudates are prone to decomposition, in term oxidation, leading to rancid products with harmful properties and a reduced food quality. The combination of high temperature, pressure, and shear stress is associated with the formation of stable protein derived radicals and short-lived lipid radicals during extrusion processing and lipid oxidation proceeds during storage.

The objective of this study was to improve the oxidative stability of polyunsaturated lipids in extrudates by investigating the effects of i) extrusion processing parameters and of ii) a coating of these extrudates with a medium chain triglycerides (MCT) oil. Therefore, corn extrudates were extruded with different feed water contents (10–22%) and lipid incorporation was analyzed with a new developed fractionated extraction protocol. The effect of microstructure, analyzed by expansion ratio and computer tomographic scans, was associated with hydroperoxides concentrations in the different lipid fractions and hexanal concentrations as an indicator for PUFA decomposition. In addition, the extrudates were coated with MCT oil and its migration was analyzed by using fluorescence microscopy and fatty acid profiles of the different lipid fractions.

The results showed that especially extrudates with a compact structure exhibit an increased amount of incorporated lipids less susceptible to oxidation. This microstructure with a low expansion was achieved by using high feed water contents. Application of an MCT coating reduced hydroperoxide concentrations in the surface lipids, significantly reduced hexanal formation during storage, and its migration inside was limited. The coating effect was strongest in porous extrudates.

In conclusion, the results of this study enable the stabilization of PUFAs in extrudates by varying the process conditions and by application of a lipid based edible coating layer.

**Flaxseed Secoisolariciresinol Diglucoside Extraction.** Yingxue Hu, University of Saskatchewan, Canada; Venkatesh Meda, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada

Flax (Linum usitatissimum L.) is an important commercial oilseed crop for industrial, food, and feed purposes. Flaxseed has a high content of oil, dietary fiber, and protein as well as bioactive compounds such as lignans. The principle lignan in flaxseed is secoisolariciresinol diglucoside (SDG), which makes up approximately 9~30 mg/g of defatted flaxseed meal. SDG has been found to have various health benefits such as anti-atherosclerosis, anti-cancer, and anti-inflammatory effects. These health benefits result in a relatively high commercial value for SDG. SDG naturally exists in flaxseed both in the form of free SDG and as a polymer composed of SDG and 3-hydroxyl-3-methylglutaric acid. Traditional SDG production involves direct organic solvent extraction of flaxseed meal and alkaline hydrolysis. Membrane filtration is used to filter the SDG polymer out and an alkaline, such as KOH, is added to hydrolyze the SDG polymer into SDG. However, since the form of the SDG polymer is unclear and inconsistent, extraction yield is usually very low and variable. The presence of alkaline also complicates further purification. In this study, organic solvent extraction will be modified by introducing simple rotary evaporation and alkaline hydrolysis will be replaced by ammonia hydrolysis. This modification avoids the high cost of maintaining membrane filtration systems and simplifies removal of alkaline. Moreover, resin purification will also be investigated. Compared to the traditional method, this modified method results in

higher SDG extraction yield and better purification efficiency with a simpler process and a lower total cost.

**Impact of Germination Processing on Chemical Properties and Functionality of Pulse Flours.** Minwei Xu, North Dakota State University, USA; Bingcan Chen, North Dakota State University, USA

Pulse grains are good sources of food ingredients in terms of protein, antioxidants, dietary fiber. Our previous research has employed controlled germination to increase the antioxidative activity of phenolics from pulses. This research focused on the impact of germination processing on the functionality of pulse flours. Chemical composition, thermal, pasting, and moisture adsorption properties of flours from chickpea (Cicer aretinium L.), lentil (Lens culinaris Merr.), and yellow pea (Pisum sativum L.) were investigated over 6-day germination. Protein content increased for pulses over germination while lentil had the highest protein content that increased from 30.65 to 33.60 g/100 g dry basis (d.b.). Two of amino acids, lysine and leucine, increased significantly during the germination of chickpea and lentil, respectively. Lipid content in pulse flours decreased over germination with chickpea being the greatest decline, i.e., from 8.00 to 5.90 g/100 g (d.b.). Total starch decreased in lentil and yellow pea flours during germination, while there was no significant change ($p > 0.05$) in germinated chickpea flours. Thermal properties of pulse flours changed slightly while pasting properties varied among pulses. The highest final viscosities for chickpea, lentil, and yellow pea flours were 1061, 981, and 1052 cP and were observed after 2, 1, and 0 days of germination, respectively. Moisture adsorption isotherms showed improved water adsorption capability after germination.

**Impact of Novel Non-thermal Food Processing Technologies of Chemical Aspects of Food.** Brijesh Tiwari, Teagasc - Agriculture and Food Development Authori, Ireland

A range of novel food processing technologies have demonstrated process efficacy in ensuring product safety, extension of shelf life, and in general a retention of key quality attributes. Novel technologies offer key advantages for advancing the preservation and quality of conventional foods, for combatting the growing challenges posed by globalization, increased competitive pressures and diverse consumer demands. However, novel processing technologies have both desirable and undesirable changes on physical, chemical and biochemical properties of foods. Extrinsic and intrinsic processing parameters employed have significant effect on macro (e.g., proteins, lipids) and micro (e.g., bioactives) nutrients. A range of novel technologies including ultrasound, high pressure, pulse electric field, light based and plasma on food chemistry and the associated degradation mechanisms with the treatment of foods will be discussed. Oxidation is one of the key mechanisms responsible for undesirable effects induced by non-thermal techniques. Research indicated that various extrinsic and intrinsic control parameters of high-pressure processing, pulsed electric field, ultrasound processing, and cold atmospheric plasma on chemistry of processed food. Proposed mechanisms and associated degradation of macromolecules, i.e., proteins, lipids, and bioactive molecules resulting in food quality changes are detailed. Understanding of key mechanisms associated with the degradation is paramount for the future uptake of novel technologies.

**Oxidomics to Explore the Radical Scavenging Activity of Olive Oil Phenolic Compounds in O/w Emulsions.** Vito Paradiso, University of Salento, Italy; Carla Di Mattia, Università degli Studi di Teramo, USA

Control of lipid oxidation in food systems is a tricky issue. One possible reason could be that the complexity of oxidation processes is disregarded and excessively simplified considering only one-two oxidation markers. Therefore, monitoring of both antioxidant effectiveness and oxidation processes often fails when coming out of laboratories. We suggest that an improved monitoring of oxidation is possible considering a larger pattern of oxidation products, deriving from a complex network of pathways taking place in food lipids. The so-called oxidomics throws a wider spot of light on the complex patterns of reactions taking place in food lipids. This research was aimed to explore how olive oil phenolic antioxidants (OPA) in o/w emulsions exert their radical scavenging activity in oil in water (o/w) emulsions, depending on the phase in which they were added. Hydroperoxide decomposition kinetics and the analysis of volatile pattern provided an outline of antioxidation mechanisms. OPA added to the water phase were more effective in slowing down hydroperoxide decomposition due to the hydrophilic radical initiator. On the other hand, OPA present in the oil were more effective in preventing radical propagation. These results can be considered representative of the different outcomes of obtaining emulsions using either phenolic-rich oils or low-quality oils together with water soluble phenolic extracts as natural antioxidant additives. The use of the former in food emulsions manufacturing is limited by the high cost of the ingredient and can be justified in high price products. On the other hand, the addition of hydrophilic antioxidants extracts could allow the use of oils with lower quality and lower price, providing increased stability to the final product. Nevertheless, the present results indicate that different oxidative outcomes should not be disregarded since the evolution of the oxidation pattern and, ultimately, the shelf-life of the product could be quite different.

**The Investigation of the Role of Processing Method on Rapeseed Oil in vitro and in vivo.** Yong-Jiang Xu, Jiangnan University, China (People's Republic); Yuanfa Liu, Jiangnan University, China (People's Republic)

The processing method is an important factor of the oil nutrition and quality. Phenolic compounds are important antioxidants in rapeseed oil. In this study, we investigated the impact of thermal pretreatment and extraction methods on the rapeseed oil based on the phenolic compounds. LC−MS/MS analysis showed that the phenolic compound contents in the microwave-pretreated oil were higher than those in the oven- and infrared-treated oils. The content of phenolic compounds in aqueous enzymatic extraction rapeseed oil is higher than that from hexane extraction, hot pressed and cold pressed oil. The cell experiment uncovered that these phenolic compounds have significant biological activities related to rapeseed oil quality. The animal experiment showed that the lipid-lowering effects in aqueous enzymatic rapeseed oil was better than the other processed rapeseed oil. This study provides the foundation for appropriate processing of rapeseed oil.

## Renewable Fuels—Technology and Market Considerations

*Chairs Alan Paine, Independent, United Kingdom; William Younggreen, Alfa Laval, USA; Bryan Yeh, American Biodiesel Dba Community Fuels, USA; Regiele Marins, Mayekawa do Brasil, Brazil*

**Alternative Feedstocks and the Synergy Between Biodiesel, Renewable Diesel and Sustainable Aviation Fuel.** Bryan Yeh, American Biodiesel Dba Community Fuels, USA

Concerns regarding global warming and incentives such as the Low Carbon Fuel Standard in California have provided the inspiration and motivation to produce fuels from low carbon intensity biomass feedstock. Demand in California alone is expected to grow by about 200 million gallons per year. While these products have different properties and characteristics, what they have in common is the use of fats and oils as feedstock, creating pressure on feedstock origination and the need to consider alternative feedstocks. This presentation will discuss the different products, how the products can be synergistic, market estimations and the role that alternative feedstock will have on the trajectory of the production of these products.

**From Frying to Flying: Sustainable Aviation Fuel (SAF) from Waste Cooking Oil.** Sergio Lima, Green Fuels Research Ltd, United Kingdom; James Hygate, Green Fuels Ltd, United Kingdom; Paul Hilditch, Green Fuel Research Ltd, United Kingdom

Hydrocarbon fuels will be central to decarbonization of aviation for the foreseeable future. There is a risk that mandates will be met by the use of fuels that are apparently renewable but entail environmental and sustainability questions, such as the use of oil from african-palm monoculture leading to undesirable land-use changes. Fuels produced by biological carbon capture seem to be less than a complete solution to decarbonization, while non-biological renewable fuels may not be an effective use of limited renewable energy. Use of waste materials to produce fuels offers a solution to these issues.

A number of routes are currently approved for production of SAF with hydrotreatment of esters & fatty acids (HEFA) allowing the conversion of oils and fat feedstocks. When waste feedstock is to be used, technical challenges include day-to-day variability of feedstock and the presence of phosphates and other catalyst-poisoning impurities. The Sustainable Aviation through Biofuel Refining (SABR) process developed by Green Fuels uses an initial transesterification reaction to remove impurities and allow consolidation of disparate supplies of feedstock into a consistent intermediate. Treatment of used cooking oil using the SABR process yields fuel satisfying ASTM criteria including distillation curve (Fig 1) and free from FAMEs (Fig 2).

**Impact of Degumming Conditions on Sorbent Efficacy to Remove Trace Elemental Contaminants in Two Common Feedstock Oils.** David Brooks, Oil-Dri Corp of America, USA; Frank Filippini, Oil-Dri Corporation of America, USA; Brandon Seiler, Oil-Dri Corporation of America; USA

The renewable diesel industry produces diesel quality fuel from hydrotreating renewable feedstock oil sources including vegetable, animal, and waste cooking oils. Conversion from triglyceride-based feedstocks to paraffin rich diesel is catalytically driven by precious metal catalysts. These catalysts are subject to various poisons including trace metals and phosphorus. Refiners employ various sorbents to drive concentrations of these catalyst poisons to sub 5 ppm levels.

Efficacy of the "bleachinG" process can be improved by application of traditional refining process techniques (pre-filtering, washing, degumming) to achieve manageable contaminant concentrations prior to entering the bleaching process. This work will focus on the impact of various degumming conditions (acid type [citric or phosphoric], % free moisture, and acid concentration) on the efficacy of a metal scavenging sorbent in two common feedstock oils (a Tallow:Corn blend and a super degummed canola).

**Methyl Esters of Fungal Biomass-derived Lipids Produced via Solid-state Fermentation of Sugarcane Bagasse and Assessment as a Blend Component in Diesel Fuel.** Mahesh Khot, University of Concepcion, Chile, Chile; Ameeta Ravikumar, Savitribai Phule Pune University, India; V Ravi Kumar, CSIR - National Chemical Laboratory, India

The present study evaluates biodiesel fuel applicability of single cell oil (SCO) produced in a consolidated bioprocessing approach via fermentation of agro-waste by fungal strain Aspergillus terreus IBB-M1. The fungus utilized raw sugarcane bagasse without any thermochemical or enzymatic pretreatment as a carbon source under solid-state fermentation conditions. Two-level statistical process optimization studies identified yeast extract (0.06 g) and temperature (40°C) as significant factors using 3 g of bagasse with a yield of 39.26 mg lipids/g dried substrate in a cultivation time of 10 days. The optimization studies suggested that running two batches, each using 3 g of the cheap, untreated, easily and abundantly available agro-waste, could advantageously produce a high overall SCO product yield of 69.7 mg in a short fermentation time of 4 days, and this was validated experimentally. The fungal SCO's fatty-acid profiling showed desirable high content of methyl oleate with the absence of polyunsaturated fatty acids (≥4 double bonds). The B5-blended biodiesel prepared from the fungal SCO's methyl esters was experimentally evaluated and compared with commercial diesel. The biodiesel-diesel blend favorably displayed higher flash point and cetane number with other fuel quality parameters (density, cetane index, distillation properties, and kinematic viscosity) comparable to commercial diesel. The result highlights the biodiesel blending potential of fungal lipids produced in solid-state fermentation. It gives necessary impetus for further process development due to process advantages in cost, energy input, wastewater generation, and operation.

**Reduction of Metals in Waste Fats, Oils, and Greases (FOG).** Jocelyn Goodwin, Applied Research Associates, USA; Ed Coppola, Applied Research Associates, USA; Chuck Red, Applied Research Associates, USA

Applied Research Associates developed the Hydrothermal Cleanup (HCU) process to remove phosphorus and metals from fats, oils, and greases. Renewable diesel refineries have very low limits on the phosphorus and metals content of feedstocks going to their hydrotreaters. The HCU process operates at conditions where it hydrolyzes phospholipids to water-soluble salts and reduces residual phosphorus content from hundreds of ppm to less than 2 ppm, all in less than two minutes of residence time. Because phospholipids are hydrolyzed, fatty acids are retained and product yield is maximized, and no waste gums or solids are produced. Metals that are present mostly in the form of soaps of fatty acids are simultaneously reduced from hundreds of ppm to less than 10 ppm total. HCU also reduces inorganic and organically bound chlorides and depolymerizes polyethylene. The HCU process allows refiners to use waste FOG feedstocks that previously have not been suitable for renewable fuel production. Two examples of contaminant reduction are shown in the table below.

| | P (ppm) | Ca (ppm) | Mg (ppm) | Na (ppm) | K (ppm) | Fe (ppm) |
|---|---|---|---|---|---|---|
| FOG Blend Feed | 201.2 | 351.9 | 12.3 | 34.7 | 42.1 | 17.5 |
| FOG Blend HCU Product | 0.4 | 1.2 | 0.0 | 0.7 | 0.0 | 0.0 |
| Used Cooking Oil Feed | 41.2 | 1.5 | 10.8 | 14.2 | 33.9 | |
| UCO HCU Product | 1.4 | 0.3 | 0.0 | 0.0 | 0.0 | |

**Use of Macauba in Renewable Fuels.** Luis Ravaglia, Soleá Brasil, Brazil

The world faces a future shortage of vegetable oils. The growing demand not only from food but also from biofuels 1G/ 2G far more exceeds the current supply. Supply growth must include new vegetable oil sources once the largest crops - Palm & Soy - faces serious challenges on environment and yield aspects. Soleá Brasil has been working for the last ten years in development a new commercial oil crop - Macauba.

Macauba has been known for many years but only recently, given Soleá's pioneer work, it has become domesticated. Accordingly, it has the ability to become a commercial crop. We will talk about Macauba characteristics and Solea's business project that will build a new global-scale vegetable oil supply chain. Sustainability, Supply Assurance&Quality as well as cost feasibility are the Pilars for this new supply chain.

## Processing Poster Session

*Poster Chair Alan Paine, Independent, United Kingdom*

**Air frying as a pre-treatment strategy for the recovery of lipophilic sinapates from lower grade yellow mustard oil.** Olamide Fadairo, University of Manitoba; Canada; N A Michael Eskin, University of Manitoba, Canada; Usha Thiyam-Hollander, University of Manitoba, Canada; Ruchira Nandasiri, University of Manitoba, Canada

The roasting of mustard seeds prior to oil extraction is a well-documented unit operation essential for the production of canolol and other lipophilic sinapates. This research investigated the effectiveness of air frying as a pre-heat treatment strategy for enhancing the recovery of lipophilic sinapates from Lower Grade Yellow Mustard seeds (LGYMS). Air frying of Lower Grade Yellow Mustard seeds (LGYMS) was conducted at temperature-time combinations of 160, 170 and 180 oC for 5, 10, 15 and 20 minutes, respectively. This was followed by removal of the oil. Extraction of lipophilic sinapates from the oil was achieved using an equal

volume of hexane and 70% (v/v) aqueous methanol. Phenolic extracts from the pre-treatment and extraction technology were quantified with HPLC-DAD. The results showed a time- temperature dependency for the recovery of major oil- soluble sinapates from lower grade yellow mustard seeds. The optimum air frying condition for the maximum yield of canolol and other major oil-soluble sinapates (RT-6.25, RT-11.50, RT-14.95 and RT-16.24) was 180 oC for 15 minutes. This information would benefit the mustard industry.

**Bioaccessibility of fish oil encapsulated by spray-drying: Influence of emulsifier and encapsulating agent.** Nor E. Rahmani-Manglano, University of Granada (UGR), Spain; Manuel Tirado-Delgado, University of Granada (UGR), Spain; Pedro J. García-Moreno, University of Granada (UGR), Spain; Antonio Guadix, University of Granada (UGR), Spain; Emilia M. Guadix, University of Granada (UGR), Spain

An increasing interest in the food industry to produce food goods containing EPA and DHA fatty acids has risen because of their beneficial effect on human health. However, the poor solubility and high prone of these nutrients to oxidation has forced researchers to develop delivery systems to incorporate them into food matrices. Microencapsulation is widely used to develop efficient omega-3 delivery systems. Whilst a lot of effort has been put into the oxidative stability of the fortified food matrices containing microcapsules, less attention has been paid to the impact of encapsulation on the digestion and bioaccessibility of EPA and DHA. Thus, in this study the effect of different emulsifiers and encapsulating agents on the bioaccessibility of omega-3 polyunsaturated fatty acids (PUFAs) encapsulated by spray-drying has been evaluated. Four types of fish oil-loaded microcapsules were produced by spray-drying 5 wt.% fish oil-in-water emulsions stabilized with enzymatically hydrolyzed whey protein concentrate (2 wt% protein) or Tween-20 (0.35 wt%). The percentages of the emulsifiers were adjusted in order to achieve the same oil droplet size. Glucose syrup or maltodextrin were investigated as encapsulating agents. The different microcapsules were then digested using the INFOGEST in vitro gastro-intestinal method. The digestion was coupled with the pH-Stat method during the intestinal phase of the digestion to assess the release of free fatty acids (FFA). The obtained results will be discussed.

**Comprehensive review on the versatile applications of centrifugal partition chromatography in Cannabis and Hemp processing.** Árpád Könczöl, Rotachrom Technologies, Hungary; Mário Nacsa, RotaChrom Technologies, Hungary; Eszter Blanár, RotaChrom Technologies, Hungary; Erika Tamás, RotaChrom Technologies, Hungary; Dóra Rutterschmid, RotaChrom Technologies, Hungary; László Óvári, RotaChrom Technologies, Hungary; Kristóf Gazda, RotaChrom Technologies, Hungary; Gergő Dargó, RotaChrom Technologies, Hungary

Centrifugal partition chromatography (CPC) is a preparative countercurrent separation technique that does not require any solid support. Thus, in CPC both the stationary and the mobile phases are liquids and resolution is simply governed by the partitioning of solutes between these immiscible liquid phases. In practice, one of the two phases is immobilized by a strong centrifugal force inside a rotor, while the other one containing the sample to be purified is pumped through the rotor. Due to its loading capacity, flexibility and cost-effectiveness, CPC has been extensively used for the lab-scale to the industrial-scale fractionation and purification of natural products.

In this presentation, CPC-based solutions to the purification requests emerging in the Cannabis industry will be reviewed through case studies representing the diverse combinations of input materials and purification tasks depicted in Figure 1: (i) pilot and industrial-scale removal of Δ9-tetrahydrocannabinol (Δ9-THC) from crude cannabis extracts and distillates; (ii) pilot and industrial-scale isolation of cannabidiol (CBD) from distillates and mother liquor; (iii) pilot-scale isolation of cannabinol (CBN) from mother liquor and synthetic reaction mixture; (iv) pilot-scale isolation of cannabigerol (CBG) from a fermentation broth; (v) lab-scale isolation of cannabidiolic acid (CBDA) by pH-zone refining from a supercritical fluid hemp extract; (vi) pesticide removal in the isolation of CBD from hemp extracts by a computer-aided approach. Crucial steps of CPC method development, such as solubility testing, solvent system screening, finetuning and recycling, and isotherm measurement will be discussed in detail.

**Electrostatic conditioning of canola oil.** Li Zhou, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada

In machine shops electrostatic "oil cleaners" are used to remove particulate material (wear particles, soot, varnish and dust) from non-polar working fluids and low dielectric constant industrial oils. Edible oils are also non-polar fluids with low dielectric constants and typical contaminants (e.g., phospholipid, free fatty acid, peroxides, seed particles) in unrefined oil are polar particles. In this study, pilot scale tests (15 liters) were conducted on unrefined canola oil using a commercial electrostatic industrial "oil cleaner" (typically used for cleaning oil in a machine shop). Unrefined canola oil was passed through the "oil cleaner" and a rapid, single pass, reduction of free fatty acids (64.4%), b-carotene (11.8%), and peroxides (33.7%) was observed. After canola oil was repeatedly circulated through the electrostatic field for 30 cycles 74.5% of phospholipid present was removed. Canola oil quality analysis indicated that the electrostatic field did not alter canola triglyceride composition. Finally, mass balance of input crude

oil and output refined oil indicated negligible neutral oil (0.37%) losses during refining.

**Fermentation of 28 Barley and 12 Oat Cultivars.** Timothy Tse, University of Saskatchewan, Canada; Daniel Wiens, University of Saskatchewan, Canada; Jianheng Shen, University of Saskatchewan, Canada; Martin Reaney, University of Saskatchewan, Canada

Ethanol production is projected to surpass 130 billion litres/year worldwide by 2024. The use of cereal grains for ethanol production has proliferated due to its positive environmental impact including renewability and reduction of greenhouse gas emissions. In North America corn and wheat are primary feedstocks used for fuel ethanol production. Furthermore, alcoholic fermentation of wheat can also produce a valuable nootropic coproduct, a-glycerylphosphorylcholine (a-GPC). Extraction of this metabolite can provide producers an additional source of revenue. However, ethanol and a-GPC yields differ among different grains and cultivars. As barley and oat production are increasing in Canada it is potentially beneficial to investigate these cereal crops as an alternative feedstocks for ethanol production. In this study, 28 cultivars of barley and 12 of oats were subjected to Saccharomyces cerevisiae fermentation. Ethanol and organic solute accumulation were followed during fermentation (e.g., a-GPC). Fermentations were sampled every 24 h until 72 h. Barley cultivars, on average, produced significantly higher amounts of ethanol (72.73 $\pm$ 3.42 g/L), and $\alpha$-glycerylphosphorylcholine ($\alpha$-GPC) (1.38 $\pm$ 0.22 g/L) than oat cultivars (59.37 $\pm$ 6.86 g/L and 0.63 $\pm$ 0.12 g/L, respectively). Specific barley cultivars accumulated more than 78.48 g/L (CDC Clear) ethanol and 1.81 g/L $\alpha$-GPC (CDC Cowboy). Consequently, barley appears to be a comparable contender feedstock for bio-ethanol production. The additional production of $\alpha$-GPC can add considerable value to these cereal crops as well.

**Formation and Physical Stability of Hazelnut Oil Nanoemulsions.** Selvi Secil Sahin, Ankara University, Turkey; Ayse Nur Akpınar, Ankara Unıversity, Turkey; Aziz Tekin, Ankara Unıversity, Turkey; Cansu Ekin Gumus, Ankara Unıversity, Turkey

Hazelnut oil, a considerable part of international industry, has not been adapted to nanoemulsion technologies, yet. The main purpose of this study is to produce nanoemulsions of hazelnut oil, a product that demonstrates cardiovascular benefits in human diet, to improve its bioavaliablity and stability. Emulsions were prepared with 2% emulsifier (Tween 80 or whey protein isolate) and 10% hazelnut oil using microfluidizer at 4–14 kpsi pressure. The zeta potential and droplet size of the samples were measured to investigate the effect of environmental factors after exposure to various conditions, such as pH (2 to 8), temperature (30 to 90°C), salt concentration (0 to 500mM NaCl), for 24 hours. Moreover, the changes during in vitro digestion and free fatty acid release were investigated. Droplet size decreased steadily with increasing pressure and number of passes (Figure 1). The use of 2% emulsifier ensured the most ideal size for both whey protein isolate (WPI) and Tween 80. Even though the particle diameter of the WPI emulsions increased at pH 5 appreciably and nanoemulsion became unstable through gelling, Tween 80 emulsions remained fairly stable (Figure 2). Especially at temperatures above 70°C in presence of 150 mM NaCl, droplet diameter increased for WPI emulsions. Nevertheless, there were no such changes for Tween 80 emulsions. During in vitro digestion, droplet size increased at stomach phase in both type of emulsions. As a result, it is determined that WPI emulsifier provides a weaker physical stability to hazelnut oil nanoemulsions than Tween 80, yet has similar strength in free fatty acid release.

**Geographical variation of polyphenols, volatile aroma compounds and haze level present in instant black tea powder produced using broken mixed fannings.** Umesh Rajapakse, Wayamba University of Sri Lanka, Sri Lanka; Chamila Jayasinghe, Wayamba University of Sri Lanka, Sri Lanka; Akila Dalpathadu, Hayleys Global Beverages (Pvt), Ltd, Sri Lanka; Darshika Pathiraja, Wayamba University of Sri Lanka, Sri Lanka; Sarath Nissanka, University of Peradeniya, Sri Lanka

Refuse tea, industrially recognized as Broken Mixed Fannings (BMF) is the most economical raw material that can be used to manufacture instant black tea powder. The commercial interest lies in determining the ideal geographical regions to source BMF to produce instant tea with the desired quality. This study aimed to determine the effect of tea growing elevation on quality parameters of BMF and instant tea produced from BMF. Instant tea was prepared from BMF obtained from eight tea estates from the three growing elevation categories in Sri Lanka i.e., high grown (HG) ( > 600m MASL), mid grown (MG) (300m-600m MASL) and low grown (LG) (< 300m MASL).

Total polyphenol content (TPC) in both instant tea and BMF in HG regions were significantly higher ($p < 0.05$) than MG and LG regions. TPC of instant tea was significantly higher ( $p < 0.05$) than that of BMF. Haze value of instant tea from HG region was also significantly higher than that of MG and LG regions. A significant positive correlation ($p < 0.05$, $r = 0.77$) was also found between TPC and haze value in instant tea.

Analysis of the aroma profile by HS-SPME/GC-MS revealed that 100 to 150 volatile compounds are present in the headspace of each instant tea and BMF samples. Major aroma compounds present in Ceylon black tea such as (Z)-3-hexenol, linalool, geraniol, phenylacetaldehyde and 2-phenylethyl alcohol were detected in BMF, but only a few of those compounds such as phenylacetaldehyde,

2-phenylethyl alcohol and benzyl alcohol remained in instant tea. Aroma profile analysis showed that the most important volatiles in tea have been lost due to evaporation and therefore possibilities exist to produce aroma condensates that resemble the aroma of Ceylon black tea as a byproduct when manufacturing instant tea from BMF.

**Healthy strategy to improve stability of chocolate: Delaying fat bloom by introducing oleogels.** Guoqin Liu, South, China University of Technology, China (People's Republic); Linlin Li, South China University of Technology, China (People's Republic); Zheng Guo, Aarhus University, Denmark

Fat bloom is the most common and serious quality flaw in the chocolate industry. As novel alternatives of solid fat, oleogels with different gelation mechanisms (monoglyceric stearate, β-sitosterol+lecithin and ethyl cellulose oleogels) are used to partially replace cocoa butter in dark chocolate (DC). The purpose of the present work is to investigate the effect of oleogels on the physical and bloom stability of chocolate. The triacylglycerol (TAG) composition and the physicochemical properties (whiteness index, mechanical strength, thermal behavior, and polymorphism of crystals) of oleogel-based chocolates were evaluated during both isothermal and fluctuating storage of 60 days. It was observed that the highly unsaturated TAGs comprised approximately 22.25% of total TAGs in the oleogel-based chocolates, which was significantly higher than that of DC. The formation of fat bloom was delayed in oleogel-based chocolate by assessing whiteness index and polarized light microscopy images. Under fluctuating temperature, β-sitosterol+lecithin oleogel-based chocolate showed lowest whiteness index value of 33.48, while DC showed highest whiteness index value of 52.82. The hardness of DC experienced a more significant change (from 10781.91 g to 25730.52 g) than that of oleogel-based chocolate due to fat bloom formation. X-ray diffraction and differential scanning calorimeter results showed that oleogel-based chocolates exhibited high thermal and polymorphic stability during the isothermal and fluctuating storage conditions. These findings demonstrated a positive effect of oleogels with different gelation mechanisms on delaying fat bloom in chocolate. This work will help to enhance the understanding of the role of oleogelation process on the stability of chocolates products.

**Implementation of chemical refining for the mitigation of 2-MCPDE, 3-MCPDE, and glycidyl esters: Promising pilot plant results.** Sergio Oey, Wageningen University, Netherlands; Ine van der Fels-Klex, Ine van der Fels-Klerx, special professor, Wageningen University, Netherlands; Vincenzo Fogliano, Wageningen University, Netherlands; Stefan P.J. van Leeuwen, Wageningen University, Netherlands

Esters of 3-monochloro-1,2-propanediol (3-MCPDE) and 2-monochloro-1,3-propanediol (2-MCPDE), and glycidol (GE) are processing contaminants that can be found in refined fats and (vegetable) oils. Recently, the European Commission has implemented new maximum limits for GE, and the sum of 3-MCPDE and 3-MCPD in vegetable oils and fats. This fuels the growing urge and necessity of oil refineries for a refining method capable to minimize both the 3-MCPDE and GE concentration in their final product.

Physical refining has become the standard refining method for the majority of vegetable oils. However, physical refining often lacks the potential for the mitigation of 2-, and 3-MCPDE. Therefore, experiments were held to explore the potentials of chemical refining, providing a viable mitigation strategy ready for adoption by the industry. Experiments were performed in our pilot plant which can hold a 100 kg of oil (Fig. 1). The pilot plant provides a good simulation of the full-scale refining process while accommodating a variety of experimental conditions such as the amount of bleaching earth, temperature, and vacuum pressure. The experiments also explored the intrinsic effects of neutralization and the effect of water washing prior to the refining process. While in operation, sample collection from the pilot plant can be done at any given moment. Quantification of 2-, 3-MCPDE, and GE were performed with an in-house validated method based on the AOCS Cd 29a-13 official method.

Compared to a single physical refining control, the chemical refining method achieved a final concentration of respectively 0.78 (–52%), 0.42 (–49%), and 0.99 (–73%) mg/kg for 3-MCPDE, 2-MCPDE, and GE in organic palm oil. These experiments have shown that chemical refining still has a great potential, especially for the simultaneous mitigation of 2-, 3-MCPDE, and GE.

**Online FTIR analysis for process control of biofuel production.** Jonathon Speed, Keit Spectrometers, United Kingdom; Stephanie Wood, Keit Spectrometers, United Kingdom

The emergence of biofuels (including fatty acid methyl ester [FAME] based biodiesel, hydrogenated vegetable oils for diesel or jet fuels, and fermentation of bioethanol) is an exciting development and potential route to much more sustainable industry over the next few decades. These developments are also ideally suited for "Industry 4.0" and advanced process control – optimising processes, improving efficiency and reducing costs. Conventional analysis techniques (such as pH and temperature probes, flow meters etc…) can give limited information in real time, but do not measure chemical concentrations directly. Offline analysis (such as chemical assays, titrations or HPLC/GC-MS) give detailed chemical breakdowns of a process, but are slow, cumbersome and expensive.

Here we present the initial work of a static optics FTIR spectrometer for on-line and real time analysis of biofuel production from natural product feedstocks. We show the detection of FAMEs in triglyceride feedstocks, whether from vegetable oils or animal fat. We also show the measurement of methanol and glycerol as well as the catalyst used in transesterification production of FAMEs from triglycerides. We briefly explain the difference between static optics FTIR and conventional FTIR and how it is advantageous in manufacturing industries.

We will also show the monitoring of a complete transesterification reaction, and how on-line analysis can be used to better control and improve the efficiency of the process.

**Polyphenols from de-oiled lower grade yellow mustard meal: Effect of enzymatic oxidation.** Afra Imran, University of Manitoba, Canada; Ruchira Nandasiri, University of Manitoba, Canada; Rotimi Aluko, University of Manitoba, Canada; N A Michael Eskin, University of Manitoba, Canada

Lower grade yellow mustard meal (LGYMM), a by-product of the mustard milling process, has potential as a value-added product in the food, feed, and agro-allied industries. Enzyme assisted extraction has several benefits including higher product yields, reduction in extraction time, lower energy utilization, and minimization of solvent use. The under-utilized by-products of mustard can also be treated with enzymes for the production of oxidized phenolic compounds. The enzymatic oxidation of sinapic acid (SA) in mustard meal was investigated by treating with horseradish peroxidase (HRP) and tyrosinase (TR) using a module system. HPLC analysis of pure SA treated with both enzymes resulted in the formation of two main compounds (1 and 2). Interestingly, sinapine in a methanolic extract of LGYMM was catalyzed significantly ($p > 0.05$) by HRP and TR into a number unidentified oxidized end products. SA released after the alkaline hydrolysis of LGYMM was also converted into Compound 1 and Compound 2 by HRP and TR. The kinetics of enzymatic reaction showed that HRP has a significantly higher catalytic efficiency (CE= 0.348), reaction velocity (Vmax= 3.48) and Michaelis-Menten constant (Km= 604.56) than TR (CE= 0.044, Vmax= 0.44, Km= 201.60). The results of this study found SA and sinapine were efficiently converted to oxidized enzymatic end products with potential as precursors for canolol (a strong antioxidant). Therefore, in-situ enzymatic oxidation has potential for adding value to the processing of LGYMM.

**Reducing glycidyl esters in edible oil with Silica.** Chelsea Grimes, W R Grace & Co, USA

Glycidyl esters (GE) in edible oil are known carcinogenic and mutagenic process contaminants that have recently been subjected to European legislation. In order to reduce glycidol concentrations in one pass RBD oil to proposed regulatory limits, we have studied a silica-based material at different temperatures, contact time, dosages, and initial GE concentrations without adversely affecting oil quality. Our poster will outline properties of this novel material as well as catalytic performance in multiple oil matrices. We will also present a kinetic analysis of this reaction to incorporate this material into existing oil refining technologies and to ultimately remove the necessity for secondary refinement.

**The Impact of pH on the Extraction of Major Sinapates from Mustard Seed.** Thu Nguyen, University of Manitoba, USA; Ruchira Nandasiri, University of Manitoba, Canada; Usha Thiyam-Hollander, University of Manitoba, Canada; N A Michael Eskin University of Manitoba, Canada

Mustard seed is found worldwide as a cultivated plant species which has a noteworthy agronomic value due to its high protein, oil and phenolic content. Mustard has been reported to contain more phenolic compounds than other oilseeds, which have shown interesting results in various target specific biological activities. The most noticeable compounds that are found in mustard is sinapates, which have a significant influence on radical scavenging activity. Many researches have been proceeded to optimize the extraction condition of major sinapates in mustard seed. However, few studies investigated the efficacy of electrostatic nature of sinapates in its extraction. The current study investigated the changes in pH on the extractability of major sinapates in different mustard varieties including oriental (Brassica juncea), black (Brassica nigra) and yellow (Sinapis alba). Experiments were proceeded with whole seeds and grounded seeds using acidified-pressurized, neutralized-pressurized and alkaline-pressurized wet extraction. From the initial data, heat treatment and pH had a significant effect ($p>0.05$) on the major sinapates of mustard seed extracts. The optimized parameter for acidified-pressurized wet extraction was found to be with 10 min of sautée time ($p>0.05$). Further studies on crushed seed at neutralized-pressurized and alkaline-pressurized wet extraction would provide the complete understanding of electrostatic nature of sinapates.

***Abstracts for the following Processing presentations were not provided:***

**Last Improvement in Preparation Equipment.** Anibal Demarco, Desmet Ballestra, Argentina

**Energy Saving in Crushing (Soybean and Rapeseed).** Anibal Demarco, Desmet Ballestra, Argentina

**Increasing Food and Feed Safety Through Cleaning of Grain and Oil Seeds.** Reviewing Different Cleaning Applications. Robin Schneider, Buhler, USA

**Trends in Solvent Extraction.** Allen Ost, Crown Iron Works Co, USA

## Proteins And Co-Products

*Division Vice Chair: Lamia L'Hocine, Agriculture & Agri-Food Canada, Canada*

### Application of Advanced, Novel, and Clean-Tech Processing for the Preparation and Utilization of Plant Proteins for Foods—Part 1

*Chairs Mehmet Tulbek, AGT Foods Research & Development Centre, Canada; Md Mahfuzur Rahman, Iowa State University*

**3D Printing of Plant Based Foods.** Nesli Sozer, VTT Technical Research Centre of Finland, Finland; Martina Lille, VTT Technical Research Centre of Finland Ltd, Finland

3D food printing is an emerging and developing technology where there is active research going on. It has been developed and initiated mainly for confectionery industry. Today's modern consumer has increased its expectations from healthy, nutritious balanced food to have elements of design, pleasure, playfulness together with active participation where self-production would ultimately provide customization or personalization. The biggest challenges associated with 3D food printing is the selection of raw materials, their rheological properties, shape and structure accuracy, material memory, compatibility with traditional food processing technologies and fast printing technologies. This presentation will give an overview on novel plant-based food design via 3D printing.

**Dry Fractionation for Sustainable Production of Legume Ingredients.** Maarten Schutyser, Wageningen University, The Netherlands, Netherlands; Regina Politiek, Wageningen University, The Netherlands; Netherlands

Conventional plant-based ingredient manufacturing relies on wet processing with thermal treatments to obtain purified ingredients that enrich foods, while at the same time having low ANF levels. However, wet processes present several drawbacks among which copious energy and water consumption, use of chemicals, while desirable micronutrients are washed out and macronutrients (such as proteins) are modified leading to loss of native functional properties. Dry fractionation (DF) of dry harvested pulses has been proposed as a more sustainable way to produce enriched functional ingredient fractions. It employs combined milling and dry separation and is estimated factor 7 more energy efficient compared to wet processing1. An important advantage of DF is that native properties of proteins are preserved, and valuable components are retained in the matrix. However, due to the mild processing the pulse fractions produced still contain undesired naturally present ANFs (influencing digestion and absorbance of micro- and macronutrients) and beany flavour components (affecting consumer acceptability), limiting their direct application in foods. Application of an additional soaking or heating treatment to remove or deactivate undesired compounds is possible, but often leads to extreme modifications, leaching or/and even damaging of the important nutrients and cancels out the positive effect of using a DF enrichment process in the first place. An alternative is the use of solid-state fermentation being a more sustainable and natural approach to remove undesired components2.

In this presentation we will give an overview of our research carried out over the last 10 years at our laboratory focusing on the development of dry fractionation of legumes. We will highlight amongst others the use of electrostatic separation technology being an alternative to air classification and the use of fermentation to enhance nutritional value of air-classified chickpea.

1 Schutyser, M. A. I., Pelgrom, P. J. M., van der Goot, A. J. & Boom, R. M. Dry fractionation for sustainable production of functional legume protein concentrates. Trends Food Sci. Technol. 45, 327–335 (2015).

2 Xing, Q., Dekker, S., Kyriakopoulou, K., Boom, R. M., Smid, E. J., & Schutyser, M. A. I., Enhanced nutritional value of chickpea protein concentrate by dry separation and solid-state fermentation. Innov. Food Sci. Emerg. Technol. 59, 102269 (2020).

**Effects of High-power Sonication on the Extraction and Structure- functions of Protein from Defatted Soy Meals.** Md Mahfuzur Rahman, Iowa State University, USA; Buddhi Lamsal, Iowa State University, USA

High power sonication (HPS) is an advanced, novel, and clean technology that can be used to process plant proteins. This study measured the influences of important process parameters on yields, molecular structure, and functionalities during HPS- assisted alkali extraction of protein from defatted soybean flakes and flour. HPS power densities at 45–720 J/mL, substrate: water ratios at 1:8, 1:10, and 1:12 (w/v), and extraction durations of 15- and 30- min were evaluated for extraction yield. Then isolates (SPI) were prepared by sonicating flakes (1:12) at 360 J/mL and 720 J/mL, and flour (1:8) at 180 J/mL and 360 J/mL; followed by extracting for 15- and 30- min at 60° C, and 5 min at room temperature with pH 8.5; and precipitating at pH 4.5. Isolates had >90% protein and were analyzed for structural changes and functional properties. A 4- min HPS of flakes at 720 J/mL showed the highest isolate yield ~85% at 60°C for 15 min extraction and ~80% at room temperature, whereas unsonicated flour yielded yield ~70% at 60°C for 30 min extraction. There were no changes in the SDS-PAGE profiles; FTIR and Intrinsic

fluorescence of SPIs also showed similar peaks and irrespective of HPS, but the different intensity with the highest intensity for room temperature extracted samples. The 4- min HPS of flakes showed lowered intensity and lesser free SH groups in the SPIs then 2 min; similar trends also found in flour. This means there was some aggregation during the longest HPS exposures. All the SPIs showed U-types' solubility curve and increased in the solubility and hydrophobicity at pH 7. Other functional properties, for example, foaming, emulsification, and gelation, are expected to be modified by HPS. Therefore, HPS enhanced protein the extraction and isolate production from plant-based substrates and modify functional properties.

**Enzymes as Structure-building and Structure-breaking Technologies Are a Powerful Tool to Address the Unique Challenges in Plant-based Dairy, Meat Alternatives, and Plant Protein Fortification.** Cynthia Machado, Novozymes NA, USA; Craig Sherwin, Novozymes North America, inc., USA

A series of studies will be shared illustrating the principles of building and breaking of structures in plant-based substrates by selecting the optimal enzyme products. These include:

- Identifying the ideal endopeptidases for increasing solubility of pea and rice protein
- Optimizing a viscous but smooth texture in cultured plant-based dairy products
- Increasing moisture and oil retention in plant-based meat alternatives
- Building macromolecular networks in extruded proteins with optimal texture
- Maintaining baked volume and bread-texture when fortifying with pulses

**Overview of Extrusion Technology Solutions for Production of Plant Protein Meat Analogs.** Brian Plattner, Wenger Manufacturing, Inc., USA; Natasa Taseski, Wenger Manufacturing, Inc., USA

Population growth and sustainability concerns have driven interest in alternative solutions to the protein demand. A demand arising from protein malnutrition faced by developing nations, and overconsumption in developed nations. Extrusion technologies, by transforming plant-based proteins into texturized ingredients that mimic meat structure, have and continue to play a big role in both meeting the demand for protein and reducing the burden on the planet from factory farming. There are several extrusion technologies that may be used to transform ingredients into desirable textures, these include traditional extrusion, high moisture extrusion, and powerheating technologies. Each technology allows for the production of hybrid, combinatorial or next generation analogs. The main factors to consider in choosing which method is suitable include process requirements, desired texture, raw materials, capacities and scalability, economics, and supply chain considerations. This overview aims to provide fundamental understanding of extrusion technologies and principles as they apply to value addition to agricultural products and production of textured plant-based meat analogs.

**Ultrasonics as a Tool to Characterize Meat Analogues In-real-time During Extrusion.** Reine-Marie Guillermic, University of Manitoba, Winnipeg, Canada; Filiz Koksel, University of Manitoba, Winnipeg, Canada; James House, University of Manitoba, Canada; Mehmet Tulbek, AGT Foods Research & Development Centre, Canada; Siwen Luo, University of Manitoba, Canada; Nasibeh Sinaki, University of Manitoba, Winnipeg, Canada; Sébastien Kerhervé, University of Canada, Canada; Anatoliy Strybulevych, University of Manitoba, Winnipeg, Canada; John Page, University of Manitoba, Winnipeg, Canada

Plant-based meat analogues are gaining interest among food industry and health-conscious consumers in the recent years. Producing plant-protein foods that possess meat-fiber-like structure and an appreciated texture is however challenging. Meat analogues are commonly produced via extrusion process, using a long cooling die in which the proteins align to form the typical meat-fiber structure. Monitoring processing conditions (such as screw speed, moisture, and barrel temperature) precisely and having more insight on the fundamental mechanisms during extrusion that lead to a "good" meat analogue are necessary to improve the sensory properties of these foods. In this study, a non-destructive in-line quality control tool utilizing low-intensity ultrasound waves was used to study the extrudate properties both in the cooling die during texturization and right at the die exit on the production line. Ultrasonic properties were linked to the texture properties of extrudates (hardness parallel and perpendicular to extrusion direction) measured with a texture profile analyzer, and the density of extrudates measured using a gravimetric method. The attenuation of the ultrasonic wave and the phase velocity depend on the presence or absence of texturization, and/or of the presence of bubbles, in the final product. Online ultrasonic characterization during extrusion is a promising quality control tool that can potentially assess material properties non-destructively in real time, and therefore prevent any downtime during production.

## Application of Advanced, Novel, and Clean-Tech Processing for the Preparation and Utilization of Plant Proteins for Foods—Part 2

*Chairs Md Mahfuzur Rahman, Iowa State University, USA; Mehmet Tulbek, AGT Foods Research & Development Centre, Canada*

**Effects of Enzymatic Hydrolysis on the Composition, Structure, Functional and Biological Properties of Almond Protein.** Fernanda Furlan Goncalves

Dias, University of California, Davis, USA; Juliana Leite Nobrega de Bell, UC Davis, USA

The need for sustainable protein sources has prompted research on several plant-based protein matrices. The effects of enzymatic hydrolysis on the composition, physicochemical, structural, and functional properties of almond protein-rich extracts (skim) were investigated. Proteins were extracted from almond flour using environmentally friendly strategies: aqueous (AEP) and enzymatic assisted (EAEP) extraction processes. Except for the use of 0.5% of protease in the EAEP, extraction parameters were similar for both processes (pH 9.0, 50 ºC, 1:10 solids-to-liquid ratio, and 60 min). The EAEP skim had higher protein content with an improved amino acid composition (less limiting amino acids). In addition, the use of enzyme to assist the extraction resulted in the production of smaller peptides, due to partial hydrolysis of amandin, and a more unordered protein secondary structure. Excepted at pH 3 and 4, the use of enzyme increased the absolute zeta potential value while a decrease in the surface hydrophobicity was observed. Hydrolyzed skim proteins had similar solubility, emulsification, and foaming properties to the AEP skim proteins, except at or closer to the isoelectric point (pH 5.0) of almond proteins. At pH 5.0, EAEP skim proteins had higher solubility (47 vs 23%), emulsification capacity (492 vs 402 g oil/ g protein), emulsification activity index (17.4 vs 8.4 m2/g), foaming capacity (23 vs 11%) and foaming stability (14 vs. 5%) compared with the AEP skim proteins. Enzymatic hydrolysis also improved the antioxidant (from 35 to 72% of ABST inhibition) and anti-hypertensive properties (from 34 to 78% of ACE inhibition) of the extracted protein. These results indicate that moderate hydrolysis significantly improved the functional and biological properties of almond protein. These results indicate that almond protein hydrolysates hold great potential for applications as ingredients in food formulations and nutraceutical applications.

**Fungal Fermentation: Exploring Potential Benefits for Pea Proteins.** Camille Massmann, South Dakota State University, USA; Mark Berhow, USDA ARS NCAUR, USA; William Gibbons, South Dakota State University, USA; bishnu karki, South Dakota State University, USA

Air-classified protein products are gaining popularity for their conserved native functionality and environmental benefits. However, air-classification of yellow peas has a few challenges such as low purity products, and off flavors. A commonly recognized source of these off characteristics are saponins, compounds produced as secondary metabolites in response to pathogens or environmental stressors.

We believe that fungal fermentation has the potential to improve quality and broaden the applications of air-classified pea proteins (APP) in food markets. This project aims to: (1) determine whether APP can support microbial growth and (2) study alterations in composition of APP after fungal fermentation.

This study included a fermentation of APP for 120 hr with daily sampling. Fermentation was performed with Aspergillus niger, Aspergillus oryzae, Neurospora crassa, Rhizopus microspores var. Oligosporus, Trichoderma reesi, and Aureobasidium pullulans. Each flask was prepared with a 10% solid loading rate by dry weight basis. Flasks were incubated for 5 days at 30°C at 150 RPM. Fermented material was analyzed for total phenolic content (TPC), saponin, mass balance, dietary fibers, and crude protein.

Results indicate that pea protein can support the growth of all fungi tested. In terms of protein, T. reesi produced the greatest increase in concentration of solids protein at 120 hours of fermentation. A. pullulans and A. niger significantly increased the protein concentration in the supernatant, suggesting an increase in protein solubility during fermentation. Longer fermentation times significantly decreased mass recovery for all organisms except R. oligosporus. Varying results were observed for TPC, where a maximum increase was achieved with pea proteins fermented by A. niger after 120 hr (14-fold increase over unfermented control). Saponin and dietary fiber data are currently pending analysis. Overall, these results demonstrate potential benefits of microbial fermentation processes for new and improved plant proteins, specifically in underutilized crops like yellow peas.

**Industrial Protein Modification Enzymes, as a Tool for Clean-labeled Plant-based Protein Foods.** Shotaro Yamaguchi, Amano Enzyme Inc., Japan

Increasing world population is calling for the depletion of food resources. In order to maintain a sustainable society, plant proteins have attracted attentions because of lower energy consumption for production than animal sources. Changes in dietary preference of environment-friendly people are also accelerating the trend. Their eating habits tend to prefer clean-label, avoiding food additives. Enzymes can provide one of the solutions for the clean-labeled food processing. Most industrial enzymes are manufactured by microbial fermentation.

Protein modification enzymes is categorized into the following three aspects.

Protein hydrolyzing enzymes are used mainly to improve the flavor. Exo-type protease/peptidases release amino acids and peptides having savory umami taste. Glutaminase is included in this category. Limited proteolysis by endo-protease can give an improved protein functionality.

Since microbial transglutaminase was discovered as a protein cross-linking enzyme at Amano, it is well known that the enzyme has brought about a significant impact to food industry. Besides the huge applications in meat,

fish and dairy processing industry, it recently attracts attentions for the plant-based food processing, such as protein texturizing and emulsion stabilizing for meat alternative. Another type of this category is oxidases. Versatile laccase is being applied for protein cross-linking.

We had continued to find a new protein modification enzyme from microorganism, resulting in a discovery of protein-glutaminase and its commercialization, creating a new category of protein deamidating enzyme. It improves the protein functionalities through the increase of protein solubility, calling a larger demand for, especially, the plant-based dairy alternative and beverage having improved stability and mouthfeel.

Amano Enzyme has a comprehensive product portfolio in this field, including several kinds of Protease/Peptidase, a Glutaminase, and a new product Laccase as a protein cross-linking enzyme. Protein-glutaminase is only available from Amano in the world. All the enzymes are derived from non-genetically modified microorganisms.

**Influence of Process Conditions on Enzyme Assisted Aqueous Extraction of Protein from Whole Rapeseed Using Pectinase.** Simone Bleibach Alpiger, Aarhus University, Denmark; Milena Corredig, Aarhus University, Denmar

In the current food market there is an increasing interest in utilisation of plant proteins. However, including plant proteins in food formulations is complex. During refinement of the ingredients, unwanted contaminants are eliminated, but the processing steps affect composition, as well as techno functional properties of the proteins. This has led to increasing focus on milder extraction methods retaining more of the protein in its native state and improving the functional properties. Enzymatic treatments have been suggested as ka means to assist in the protein isolation from low-value by-products such as rapeseed press cakes. Rapeseed is the second largest produced oil seed in the world, and the oil production leaves large amounts of by-products rich in fibres and proteins, which can be further utilised as a novel protein source. However, understanding protein extraction from the raw material is crucial in order to convert the rapeseed press cake into a functional ingredient.

The aim of this research is to investigate how different process conditions during enzyme assisted aqueous extraction of protein from whole rapeseed affects the composition and colloidal structure of the protein extracts. The investigated process parameters are: enzyme dose (10 and 100 mg/g rapeseed), extraction pH (5.5 and 8.5), extraction temperature (20 °C and 60 °C) and heat treatment (80 °C for 3 s). The composition of the protein extracts were analysed with respect to protein, fat and carbohydrate concentration, and the colloidal structure were examined by determining the particle size and protein solubility. The colloidal structure were visualised by confocal laser scanning microscopy.

This study constitutes the foundation of a larger research project investigating the utilisation and extraction of protein deriving from rapeseed press cake by a combination of pectinase treatment and ultrafiltration with the purpose of creating high-value protein concentrates from low value by-products.

**New Marine-sourced Human Food Protein Isolate Extraction Technology with Potential Market Disruptive Properties.** Fred Jewett, Advance International Inc, USA; Kalalid Mentak, Advance International Inc, USA; Ernesto Hernandez, Advanced Lipid Consultants, USA

Common sources of commercial protein isolates and concentrates include whey and soybean, and to a lesser extent pulses like lentil, peas and chickpea. Their recovery methods usually involve energy intensive processing steps, in some cases harsh conditions that can affect negatively functional or nutritional or organoleptic properties. The newly patented SEAVIOR® System produces human food grade protein isolates from marine sources using mild processing conditions and allows production of protein isolate powders with nutritionally complete amino acid profile, desirable organoleptic properties, extremely low carbon footprint, no reliance on arable land, and virtually no fresh-water demand.

The described protein extraction and purification consists of grinding food grade raw fish and a countercurrent extraction process using a food grade solvent mixture. The recovered protein was filtered and desolventized at low temperature, separating out the water, oil and impurities, then dried. The high-grade fish oil extracted was also recovered in separate desolventizing steps. The resulting solvent was recovered for reuse in processing.

The protein isolates were analyzed for protein content (92% ±3%), amino acid profile, ash (5% ±2%), minerals, residual oil ( < 0.5%), moisture ( < 3%), cholesterol (0%), fiber and carbohydrates (0%). It also showed low molecular weight distribution (69% peptide fractions < 6.0 KDa) and a high level of essential amino acids including good levels of the branched chain amino acids Leucine, Isoleucine, and Valine. The isolate had over 1.0 protein quality (PDCAAS) score. The protein isolate was highly stable (estimated shelf life over 2 years), with only mild aroma and flavor. The milder processing conditions used in the recovery of this protein isolate also allowed for little or no deterioration of the protein or recovered fish oil fractions.

**Non-thermal Ultrasound Contact Drying, a Novel Method to Reduce Drying Time and Prevent Processing Damage.** Nahla Kreidly, University of Illinois at Urbana-Champaign, USA; Graciela Wild Padua, University of Illinois at Urbana-Champaign, USA

Novel plant protein products are currently of high interest due to consumers' preference over the use of animal proteins. Although the demand for plant protein-based food is increasing, many challenges are still associated with plant proteins' processing. One common challenge is the drying of protein-based foods due to the sensitivity of proteins to high heat and exposure times, affecting the product quality and nutritional value. Ultrasound (US) has become a technology of interest to the food industry due to its ability to remove water and solvents without significantly raising the load's temperature.

The mechanism of drying almond, lentil, and pea protein slurries and gels by ultrasound was investigated. Ultrasound drying of protein samples was performed with a custom-designed system consisting of a transducer box that vibrated at 40 kHz. The transducer box was submerged in a water jacket for temperature control. The drying temperature was 28C, drying time was 8 min, and the final moisture content was ~1%. Drying rate curves showed that ultrasound drying exhibits a unique drying rate curve with a long constant rate and a slight falling rate period, indicating that most drying is happening in the constant rate period. Besides, ultrasound drying offered a short falling rate period indicating the complete removal of solvent left within the matrix. This has different advantages than processes than having a long falling rate period that might cause case hardening, change in the product's characteristics, and microbiological safety.

Structural changes were investigated by CT scans. US drying formed thin films with a uniform structure, while air drying formed a semi-porous system. Ultrasound drying significantly enhanced the solubility of plant protein gels.

Ultrasound drying offers an attractive alternative for drying proteins due to its potential to preserve protein functionality, including solubility and enzymatic activity.

## Functionality of Proteins in Food and Interaction with Other Food Components

*Chairs Jiajia, Rao, North Dakota; State University, USA; Hongbing Fan, University of Alberta, Canada; Chibuike Udenigwe, University of Ottawa, Canada*

**Developing Functionally Enhanced Pea Proteins as Novel Food Ingredients.** Yonghui Li, Kansas State University, USA; Yanting Shen, Kansas State University, USA

Proteins are important food ingredients in delivering both nutritional and functional properties. There has been an increasing interest in utilizing plant proteins in various food products due to the perceptions of their health and environmental benefits. Functional properties are critical in determining the usefulness of plant proteins. However, native plant proteins, such as pea proteins, are inferior in certain functional attributes and only have limited practical applicability in foods. Our objectives were to enhance the functional properties of pea protein isolate (PPI) through different biochemical or enzymatic modifications, including acylation, glycosylation, deamidation, and crosslinking, as well as sequential modifications through combined approaches. Acetylation of PPI enhanced its water holding capacity (WHC) and gelation property, while succinylation improved solubility, WHC, and emulsification property. Sequential acetylation and glycosylation with guar gum further dramatically enhanced the WHC, emulsification, and gelation properties. Besides, sequential modification of PPI with protein glutaminase or transglutaminase, followed by conjugation with guar gum also greatly enhanced the WHC, emulsification, and gelation properties. The modified PPIs were further characterized using various analytical techniques to elucidate the molecular changes that were responsible for the functional enhancement, and in vitro protein digestibility was also evaluated. The functionally enhanced PPIs demonstrated application potential in bakery and meat products.

**Effect of Processing Conditions on Pulse Protein-polysaccharide Interactions.** Burcu Guldiken, University of Saskatchewan, Canada; Maxime Saffon, Nestle, USA; Supratim Ghosh, University of Saskatchewan, Canada; Michael Nickerson, University of Saskatchewan, Canada

Protein-polysaccharide interactions play an important role in food product development, especially food emulsions. These complexes promise enhanced functionalities in end-products compared to protein solutions alone. Therefore, in this study, interactions of yellow pea (PPI) and faba bean proteins isolates (FPI) with various polysaccharide sources (pectin, guar gum, gum Arabic (GA)) were investigated at different mixing ratios (1:1, 1:10) at room temperature and after a temperature treatment (heated to 75°C for 20 min, then cooled to 10°C), which mimic beverage processing. The zeta potential and turbidimetric analysis were followed as a function of pH (1.5–8.0) for proteins, polysaccharides, and their complexes. Isoelectric points (IEP) of guar-PPI complexes (pH 4.5) in all ratios were found similar to PPI (pH 4.7), which decreased to pH 2.5 in pectin-PPI complexes at the 1:1 ratio. Similarly, the lowest IEP was found at pH 2.1 in pectin-FPI (1:1) complexes. For PPI complexes, the highest maximum optical density (MOD) was determined 0.63 in processed GA-PPI (1:1) complexes; the lowest was found 0.26 in processed Guar-PPI (1:1) complexes. Overall, MODs of FPI complexes were lower than the PPIs. Besides, the lowest MOD for FPI complexes were detected in pectin-FPI (1:1), and the highest was processed by GA-FPI (1:1). Findings from this study showed that soluble

complexes can be obtained at the desired pH range regarding the protein and polysaccharide sources that can be used to stabilize various types of emulsion-based beverages.

**Impact of Processing on the Functionality and Protein Quality of Plant Protein Ingredients.** Michael Nickerson, University of Saskatchewan, Canada

The demand for protein ingredients is increasing at a significant rate, being driving by population growth, increased need for sustainable agriculture, urbanization, changing demographics, nutrition and emerging regulatory influencers. However, the incorporation of plant protein ingredients into food formulations can be challenging due to issues associated with flavour, functionality and nutritional value. Processing technologies such as infrared heating, extrusion, fermentation, enzymes, germination and so on, can offer a means of modify both the protein functionality and the quality, in terms of their protein digestibility corrected amino acid scores (PDCAAS). The presentation will discuss how infrared heating, fermentation and enzymatic treatments can modify the plant protein ingredients. For instance, infrared heating was shown to reduce the solubility and foaming properties of Desi chickpea flour, improve the water hydration capacity, whereas oil holding and emulsification properties remained unchanged. Processing also lead to increases in in vitro protein digestibility and in vitro PDCAAS values. The fermentation of pea protein enriched flour showed improved water and oil holding capacities, decreased solubility and variable emulsifying properties, whereas protein quality remained unchanged. And enzymatic modification of the same material led to improved water and oil holding capacities, reduced solubility and emulsifying properties, and variable foaming attributes. However, depending on the enzymatic conditions, protein quality could be improved or lowered. Significance of this research has shown the ability to tailor ingredient performance for the food industry, which could have use for product development purposes.

**Inhibitory Effect of β-glucan Interaction on in vitro Digestibility of Proteins Isolated from Lentil (Lens Culinaris) and Yellow Pea (Pisum Sativum) Seeds.** Ruth Boachie, University of Ottawa, Canada; Mieke M. Commandeur, Wageningen University, Netherlands; Raliat Abioye, University of Ottawa, Canada; Edoardo Capuano, Wageningen University, Netherlands; Teresa Oliviero, Wageningen University, Netherlands; Vincenzo Fogliano, Wageningen University, Netherlands; Chibuike Udenigwe, University of Ottawa, Canada

Interaction between food proteins and co-existing biomolecules in a food matrix can affect protein digestibility. In this study, the effect of barley β-glucan on the in vitro digestibility of lentil and yellow pea proteins under simulated static gastrointestinal conditions was investigated. The proteins were mixed with β-glucan at mass ratios of 1:0.5, 1:1 and 1:2, and subjected to simulated in vitro digestion. The interaction between β-glucan and the isolated proteins was demonstrated by the decrease in transmittance and surface charge and increase in particle size of the complexes. Brightfield microscopy showed the formation of aggregates between the biopolymers, although increased molecular size was not observed by discontinuous native PAGE. Fluorescence microscopy of the mixtures indicated that β-glucan formed aggregates with lentil protein while the interaction with yellow pea protein appeared as distinct phases of protein within the β-glucan network. The relative orientation was relayed onto the dose-independent in vitro protein digestibility, with the protein-β-glucan mass ratio of 1:2 decreasing the in vitro digestibility of lentil and pea proteins by 24.7% and 37.6%, respectively. The findings show that the addition of β-glucan either as a functional or bioactive ingredient hinders protein digestibility in a food matrix.

**Modification of Pulse-based Protein for Improved Functionality and Flavor Profile.** Jiajia Rao, North Dakota State University, USA

Consumer preference to have a healthy eating pattern has led to an increasing demand for more nutrients dense and plant-based foods. Pulse proteins are exceptional quality ingredients with potential nutritional benefits and unique functional performances, and might act as health21 promoting agents for addressing the new-generation foods. However, the utilization of pulse protein in foods has been hampered by its poor functionality and unpleasant flavor. Protein structure modification has been proved to be a useful means to improve the functionality and flavor profile of pulse protein. In this presentation, pea protein isolate (PPI) will be selected as a pulse protein representative. This talk begins with a brief introduction of hierarchical structure of PPI material to better understand the structure characteristics. It will comprehensive discussed the impact of selected physical and chemical modification on functional properties and flavor profile of PPI, which has been done in our lab in past few years. We will mainly focus on soluble complexes, maillard reaction and phosphorylation of PPI. In general, protein solubility could be improved by means of abovementioned three methods. Maillard-driven synthesis of the cross-linked PPI-gum arabic conjugates greatly improved the flavor profile and functionality of PPI. In terms of phosphorylation, sodium hexametaphosphate (SHMP) is a good candidate to form phosphorylated PPI with enhanced functionalities including foaming, emulsifying properties and thermal stability. Our results suggested that protein structure–function researches are valuable in tailoring proteins for specific functional outcomes and expanding the availability of pulse proteins.

**Oxidative Products of Peptides Due to Interaction with Peroxyl Radicals.** Ramak Esfandi, Carleton University, Canada; Apollinaire Tsopmo, Carleton University, Canada

Food-derived peptides possess various biological activities, some of which are useful in preserving foods and the management of chronic diseases. However, the activity of a peptide is affected by the matrix composed of other food components and oxidant species. The interaction of peptides with polysaccharides is well documents. In addition, peptides and proteins are also able to interact with secondary metabolites such as polyphenols. Peptides have antioxidant activities in part because they can interact with reactive oxygen species such as peroxyl radicals and hydroxyl radicals. These reactive species are formed during normal metabolisms, exposure to toxicants, storage, or food processing. By acting as peroxyl and hydroxyl radical scavengers, Peptides quench or react with oxidative species, thereby restoring the redox balance or preventing further oxidation. In this work, we were interested in determining the reaction products of five selected oat peptides in the presence of peroxyl radicals (i.e., ROO• from AAPH). The tested peptides were YFDEQNEQFR (P1), GQLLIVPQ (P2), SPFWNINAH (P3), NINAHSVVY (P4), and RALPIDVL (P5). In the presence of ROO•, peptide P3 only retained 4% of its original structure while other peptides maintained 69 – 89% of its structures. Using the HPLC method can help to identify the oxidized products of the peptides. For example, the chromatogram results showed that only one main oxidized product was present in peptide P1; meanwhile, there were four products in the case of P2. This is an interesting finding, and the determination of the structures of oxidative products from each peptide could present an insight into their radical scavenging properties.

## General Protein and Co-Products

*Chair Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada*

**Antihypertensive Effect of Enzymatically Fabricated Bioactive Peptides from Bellamia Benghalensis Protein: An in Vitro and in Silico Study.** Pubali Dhar, University of Calcutta, India

Bellamya bengalensis, an edible Mollusca, is an indigenous protein-rich food source for the tribal population. This protein-rich food have been employed to isolate short bioactive peptides by ultrafiltration technique. The antihypertensive property of the bioactive peptides was compared with a standard drug using in vitro as well as detailed in silico molecular docking study to identify the most active inhibitory peptide against angiotensin converting enzyme. These results were further rationalized by isothermal titration calorimetry (ITC) to gain further insights about the actual mechanism of action or how these peptides exert their antihypertensive property.

Bellamya bengalensis protein isolate was hydrolyzed utilizing various proteases in vitro. Excised Bellamya bengalensis protein isolates were characterized for the functional properties like protein solubility index, emulsifying property, foaming property. Small peptide fraction (< 3kD) were ultra filtered from 120min alcalase-hydrolysates and in vitro ACE-inhibitory activity was analyzed. This fraction was assessed in MALDI-TOF MS and five peptides were sequenced via de novo sequencing. Synthetic analogue of the bioactive peptide was used to understand the thermodynamics of the inhibition by checking the binding affinity of the peptide to Angiotensin Converting Enzyme by Isothermal Titration Calorimetry compared with lisinopril, and further substantiated by in silico site-specific molecular docking study.

The IC50 value of the 120 min hydrolysate ultrafiltered fraction was found to be 86.74 $\pm$ 0.575 μg/mL, while the IC50 of Lisinopril is 0.31 $\pm$ 0.07 μg/mL. The ACE-inhibitory potential of the peptides have a positive correlation with the hydrophobicity of the amino acids.

Peptides from different food matrices have been studied, and indicated as compounds with particular interest in the context of hypertension. Bioactive peptides from edible animal source reveals diverse biological activities including antihypertensive potential. These bioactive peptides with antihypertensive effects may serve as a value-added food and pharmaceutical product.

**Effect of Biopolymer Mixing Ratios and Aqueous Phase Conditions on the Interfacial and Emulsifying Properties for Lentil Protein Isolates- κ-carrageenan, -i-carrageenan Complexes.** Yingxin Wang, University of Saskatchewan Canada; Michael Nickerson, University of Saskatchewan, Canada; Supratim Ghosh, University of Saskatchewan, Canada

This study investigated the effect of pH on the formation of electrostatic complexes between lentil protein isolate (LPI) and carrageenan (κ-carrageenan: κ-C and ι-carrageenan: ι-C) at 4:1, 8:1, 12:1 protein-polysaccharide mixing ratios, and the emulsifying properties of formed electrostatic complexes at pH 6 and 3.5. Incorporation of the both carrageenan polysaccharides into the LPI solution led to the suppression of complexes being formed during a turbidimetric pH acid-titration. As mixing ratio decreased, maximum optical density (max OD) values for both LPI-κ-C and LPI-ι-C systems decreased. Coacervate emulsions made at pH 6 showed higher emulsion stability (ES) than those made at pH 3.5 for each sample. Emulsions at pH 6 also formed smaller, more uniform, and higher charged droplets, and showed lower interfacial tension. The greatest ES was found at

4:1 LPI-κ-C and LPI-ι-C emulsions at pH 6 mainly due to the higher continuous phase and emulsion viscosity.

**Extraction and Properties of Covercress, a New Pennycress-derived Cover Crop and Plant Protein Source.** Milagros Hojilla-Evangelista, USDA ARS NCAUR Plant Polymer Research Unit, USA; Roque Evangelista, USDA ARS NCAUR, USA

New field pennycress hybrids are being developed through conventional breeding techniques or gene editing to enhance protein properties. Little is still known about the chemical and functional properties of the proteins of these hybrids. In this research, two of these lines (Black, B3 and Yellow, Y1126) were evaluated for protein extractability and functional properties (solubility, foaming, emulsification). Seeds were first ground cryogenically using liquid nitrogen in a Fritch mill (final particle size 250–350 μ) and then defatted with hexane in a Soxhlet extractor until residual oil content was ≤ 0.5%. B3 and Y1126 defatted meals, with initial protein contents of 35 and 38% dry basis, respectively, were each subjected to protein extraction using our saline-based method (1: 10 w/v, 0.1 M NaCl, 2 h, 50°C) for wild pennycress. Protein yield from Y1126 (49%) was higher than those of B3 (36%) and wild pennycress (45%). Freeze-dried extracts contained 71–75% crude protein and showed high protein solubility (65–75% soluble protein) from pH 2–10, which is similar behavior as wild pennycress protein. B3 and Y1126 protein had similar foaming capacities (113–121 mL) that were largely unchanged with pH and formed stable foams ( >90% remaining foam after 15 min). Emulsifying activity indices (95–230 m2/g protein) and emulsion stability indices (13–24 min) were also similar and increased with pH. Foaming and emulsifying properties of both hybrid B3 and Y1126 proteins matched those of wild pennycress protein. This work demonstrated that the pennycress hybrid protein has desirable properties as a novel plant-based protein.

**Impact of Structure-composition on Gel Microstructures and Rheological Properties of Vicilin and Legumin-like Proteins Derived from Bambara Globulin.** Opeyemi Alabi, Durban University of Technology, South Africa, South Africa; Eric Amonsou, Durban University of Technology, South Africa, South Africa

Understanding the structure-composition and functionality of pulse proteins and their subunits is essential to facilitate their utilization as replacement for animal-based proteins. In this study, Bambara globulin was extracted and fractionated into constituent sub-fractions for structural, physicochemical, and heat-induced gel rheological characterization. Although vicilin was the major fraction of Bambara globulin, a legumin-like protein was found in relatively low quantity. Globulin revealed three major protein bands at 50, 58, and 70 kDa, similar to vicilin with predominant β-sheet structures. One basic subunit (22 kDa) was identified in the legumin fraction. Vicilin displayed the highest sol-gel transition temperatures (80 °C) followed by legumin (50 °C) and globulin (40 °C). After the gel point, the G′ and G″ of globulin showed relatively low dependency on heating time, suggesting a rapid establishment of its gel network. Vicilin and legumin displayed a more progressive establishment of their protein, with G′ increasing over the heating time. Vicilin gel consisted of a microporous structure with a small lath sheet-like structure compared to globulin and legumin. The presence of disulfide linkages in globulin and legumin, the number of protein subunits, and protein hydrophobicity appear to be the most influencing factors on gelation of Bambara globulin and its fractions and consequently their application as food protein gels.

**Physicochemical and Functional Properties of Protein Fractions Obtained from Green Pea, Lentil, and Chickpea.** Liuyi Chang, North Dakota State University, USA; Jiajia Rao, North Dakota State University, USA

In recent years, pulse proteins might act as health-promoting protein ingredients for addressing new-generation foods. In general, salt soluble globulin is the major storage protein fraction found in pulses and can be further categorized as (11S) legumin and (7S) vicilin subunits according to their sedimentation coefficients. Currently, there is a lack of systematic study on structural and functional relationships among protein fractions from different pulse resources. Protein structure-function researches are valuable in tailoring proteins for specific functional outcomes and expanding the availability of pulse proteins. As such, the amino acid composition, structure, functional attributes of a major fraction of pulse storage proteins including globulin, legumin, and vicilin isolated from green pea, lentil, and chickpea were studied. Individual protein fractions from defatted pulse flours were obtained from alkaline extraction-isoelectric precipitation and further separate legumin and vicilin by salt dissolution-precipitation method, followed by freeze-drying. Results showed that sources and fractions had significant impacts on the amino acid composition, structural and functional properties of proteins including solubility, foaming, and emulsifying properties. In general, vicilin had higher functionalities compared to legumin. For example, the foaming capacity of vicilin extended to 260.0 ± 10.14%, whereas, that of lentil legumin was only 47.5±10.81%. Regarding the impact of pulse crops on the functionality of protein fractions, lentil protein had the superior foaming and emulsifying properties, followed by green pea and chickpea.

**Ultrasound-assisted Extraction and Modification of Plant-based Proteins: Impact on Physicochemical, Functional, and Nutritional Properties.** Md Mahfuzur Rahman, Iowa State University, USA; Buddhi Lamsal, Iowa State University, USA

Ultrasonication is a green technology that can be applied to the extraction of plant-based proteins and tailoring their functionalities for various food applications. Ultrasonication can generally be applied as a pretreatment step in the conventional protein solubilization and extraction protocols for its ability to disrupt the cell-matrix, thus, improve extraction yields. In addition, high-power sonication can also be applied to modify the physical, structural, and functional properties of protein-based ingredients for food applications. The important ultrasonic parameters such as energy density, extraction duration, substrate to slurry ratio, agitation, and environmental conditions like pH and temperature impact the rate of protein solubilization and extraction yields. The ultrasound-induced cavitation leads to changes in protein secondary and tertiary structures, including aggregation and cross-linking due to oxidation. Other changes in protein include size reduction, rheology, electrical conductivity, zeta (ζ) potential, etc. These physical and structural changes result in modified ingredient functionality, especially hydrophobicity, solubility, emulsion, and foaming, and nutritional quality of protein, depending on the extent of ultrasound energy applied to the protein. Ultrasound induced protein denaturation increases the solubility compared to native protein; however, extended sonication can reduce the solubility and solubility-dependent functions. This presentation will discuss the major ultrasound process parameters and conditions for extraction and modification of plant proteins and their impact on protein structural changes and resulting physicochemical, functional, and nutritional properties.

## Lifetime Achievement Award and Lecture/Graduate Student Oral Competition

*Chairs Buddhi Lamsal, Iowa State University, USA; Janitha Wanasundara, Agriculture and Agri-Food Canada, Canada*

**Efficacy of Great Northern Beans-derived γ-glutamyl Peptides in Reducing Vascular Inflammation.** Snigdha Guha, University of Nebraska, Lincoln, USA; Kaustav Majumder, University of Nebraska-Lincoln, USA

Dry beans, such as Great Northern beans (GNB), are excellent sources of plant-based proteins and naturally occurring dietary peptides. One such class of peptides found in beans are γ-glutamyl peptides, which are known for their ability to reduce gastrointestinal inflammation via allosteric interaction with the calcium sensing receptor (CaSR). This study aimed to develop a large-scale extraction method for isolating the γ-glutamyl peptides from GNB and subsequently evaluate their efficacy in reducing vascular inflammation, which is a key contributor of pathogenesis leading to cardiovascular diseases (CVDs). The extraction method generated two GNB fractions which were evaluated for their anti-inflammatory activities in human umbilical vein endothelial cells (HUVECs). Human tumor necrosis factor-α (TNF-α: 5ng/mL, 6 h) was used to induce inflammation in HUVECs pre-treated (2 h) with different concentrations of the fractions based on their peptide content (25, 50, 100 μg/mL peptide concentration). TNF-α treatment significantly increased the expression of inflammatory adhesion molecules (ICAM-1, VCAM-1) and chemokine (MCP-1). However, the expression of these biomarkers was significantly reduced by one of the GNB fraction (bioactive fraction) at peptide concentrations starting from 50 μg/mL. Furthermore, a simulated in-vitro gastrointestinal digestion of the bioactive fraction was performed to ensure the constancy of the bioactivity, and it was found that the fraction retained its anti-inflammatory activity after gastrointestinal digestion but at much higher peptide concentration of 500 μg/mL, due to further hydrolysis of the longer peptides. The bioactive fraction consisted of di- and tripeptides, including γ-glutamyl peptides such as γ-glutamyl valine (γ-EV) and γ-glutamyl cysteine (γ-EC). LC-MS/MS analysis further quantified 53 ng/mg of γ-EV as the most abundant γ-peptides from the GNB bioactive fraction. Thus, the present study highlights the potential use of dry-edible beans in the development of functional foods for the prevention and management of CVDs.

**Expanding Plant Protein Utilization as Food and Feed Through Innovative Processing.** Keshun Liu, U.S. Department of Agriculture, USA

Incorporating plant proteins into food and feed products by partial or full replacement of animal proteins is becoming important for human health, environmental protection, and sustainability. In achieving this noble goal, we need develop processing technologies in two fronts. One is to extract and/or concentrate proteins, and the other is to effectively incorporate protein and other ingredients into final products. In this presentation, I provide a brief overview of this broad subject, with some focus on my own research on plant proteins over the years. For the first front, improved dry and wet processes have been developed. For the second front, extrusion has been a method of choice. In general, extrusion can be categorized into low- and high-moisture processes. Unlike low-moisture extrusion, high-moisture extrusion features a twin-screw extruder with a cooling die. Proteinaceous materials are processed under 45–75% moisture levels. Extrudates do not expand, show up fibrous structures, and thus resemble muscle meat. During extrusion, many complex physicochemical reactions occur, and numerous parameters are involved, making it difficult to develop a proper extrusion process. As animal proteins become

expensive and non-sustainable and as many new meatless products are fast entering markets, there is renewed interest in incorporating plant proteins into food and feed products. Innovations to the dry and wet fractionation and extrusion processes will enable continued expansion of plant protein utilization worldwide.

**Hydroxyl Radical Generated During High Power Sonication of Soy Proteins and Its Effect on Protein Structure.** Md Mahfuzur Rahman, Iowa State University, USA; Buddhi Lamsal, Iowa State University, USA

High power sonication (HPS) is shown to alter protein structure, thus, its functionality, via intermolecular interactions. This study evaluated the effects of HPS of soy proteins in aqueous medium on molecular structure. Free radicals generated during HPS, were quantitated using the 5,5-dimethyl-l-pyrrolin N-oxide (DMPO) spin trap method. Electron paramagnetic resonance (EPR) was used to identify them as mostly hydroxyl radicals, and quantified. The minimum saturation concentration of spin trap was determined to be 500 mM of DMPO in water when exposed to 5 W/cm3 ultrasound power density (PD) for 10 min; subsequently, this concentration was used for quantitating radicals in protein samples. Five aqueous soy protein systems, namely, 5% soy protein isolate (SPI), 5 % isoflavonoids removed SPI (NO-ISO SPI), subunits: 1% glycinin (11S) and 1% β conglycinin (7S) as well as soy flakes (1:10 in water), were sonicated at PD of 2.5 and 5 W/cm3. Only hydroxyl radical adducts (DMPO-OH) was detected in these aqueous systems. Highest concentration (3.68 μM) of DMPO-OH adduct was measured in 11S solution at 5 W/cm3, whereas lowest (0.67 μM) concentration was in soy flakes protein. PD 5 W/cm3 generated higher concentration of radicals in 7S subunit solution, NO-ISO SPI, and soy flakes protein, compared to sonication at PD 2.5 W/cm3. No change in the protein electrophoretic pattern was observed. However, some changes in the estimated secondary structure and tertiary structure as well as in the content of free sulfhydryl bonds and disulfide bonds were observed after HPS.

## Lifetime Achievement Award and Lecture/Graduate Student Oral Competition

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*Reviewer/Judges Rotimis Aluko, University of Manitoba, Canada; Keshun Liu, U.S. Department of Agriculture, USA*

**Nutritional Profile of Some Physically Modified and Fermented Legume-protein Ingredients.** Bibek Byanju, Iowa State University, USA; Buddhi Lamsal, Iowa State University, USA

Despite possessing a significant amount of nutrients, including dietary fibers, proteins, minerals, and vitamins, their usage and availability in legumes is impacted by the presence of anti-nutritional factors (ANFs) like phytic acid, tannins, and enzyme inhibitors. This research evaluated the effect of fermentation of some modified legume-protein ingredients with Lactobacillus plantarum and Pediococcus acidilactici on important ANFs. The physically modified (sonication or precooking) flour slurries of soybean, lentil, and green peas were inoculated with Lactobacillus plantarum and Pediococcus acidilactici at 108 CFU/mL and fermented in shake flasks for 72 h at 37°C, with shaking at 200 rpm. Total phenolic contents were significantly ($p < 0.05$) reduced by 50% for physically modified and fermented green pea, precooked green pea, precooked lentil compared to non-fermented controls. Trypsin inhibitory activity (TIA) was reduced by more than 20% for all the substrates except for unsonicated soybean and lentil fermented with L. plantarum and P. acidilactici. For precooked and fermented lentil and green peas, there was a decrease in TIA by 80%. For fermentation of physically modified soybean and lentil with both microorganisms, phytic acid content decreased but it was not significant for green pea flours. In vitro protein digestibility increased for precooked (control and fermented by both microbes) green pea and lentil; however, it did not change significantly for other substrates. Similarly, there were changes in the content of individual essential and non-essential amino acids when physically modified and fermented. In summary, the fermentation of physically modified legume flours positively influenced the non-nutritive compounds, thereby potentially improving their nutritional quality and usage.

**Spent Hen-derived Angiotensin-converting Enzyme 2 (ACE2) Upregulating Peptide Reduces Blood Pressure in Spontaneously Hypertensive Rats.** Hongbing, Fan, University of Alberta; Canada; Jianping Wu, University of Alberta, Canada

Spent hens are laying hens that reach the end of the egg-laying cycle. Despite being treated as a major byproduct in the egg industry, spent hens are a rich protein source that can be bio-transformed into bioactive peptides with enhanced health-beneficial effects for value-added applications. We previously reported a spent hen muscle protein hydrolysate digested by thermoase (SPH-T) exhibiting a great blood pressure-lowering activity in spontaneously hypertensive rat (SHR, an animal model of human essential hypertension); upregulated plasma and vascular ACE2 levels were detected in the SPH-treated animals, indicating that its antihypertensive effect may be due to ACE2 upregulation, as well as the presence of ACE2 upregulating (ACE2u) peptides therein. Thus, the present study aimed to identify ACE2u peptides from SPH-T and then investigated their antihypertensive effects in SHR. As a result, four ACE2u peptides were identified varying in peptide length of 3–25 amino acids

using the conventional activity-guided fractionation. The one with the highest in vitro ACE2u activity (VVHPKESF) was therefore orally administrated to the animals at a dose of 15 mg/kg/day body weight for a period of 18 days. Results showed that VVHKPESF significantly reduced blood pressure, possibly through multiple mechanisms involving unregulated circulating and aortic ACE2 level as well as amelioration of vascular inflammation and oxidative stress. This study reported for the first time that an ACE2u peptide identified using the conventional activity-guided fractionation exerted antihypertensive effects in SHR, which may support the role of ACE2 upregulation as a novel mechanism regulating hypertension by bioactive peptides. the definite article More (Definitions, Synonyms, Translation)

## Non-Food Applications of Proteins

*Chairs Nandika Bandara, University of Manitoba, Canada; Bishnu Karki, South Dakota State University, USA*

**Canola Protein-based Films Prepared with TEMPO Modified Nanocrystalline Cellulose for Sustainable Food Packaging.** Thilini Dissanayake, University of Manitoba, Canada; Tizazu Mekonnen, University of Waterloo, USA; Senaka Ranadheera, University of Melbourne, Australia; Nandika Bandara, University of Manitoba, Canada; Boon Peng Chang, University of Guelph, Canada

The demand for biodegradable food packaging is increasing due to the negative environmental impact of petroleum-based resins. Canola protein is a potential biopolymer candidate for developing biodegradable packaging but suffers from poor mechanical and barrier properties. The addition of nanomaterials could provide solutions to the weak functionality of biopolymers. This study aimed to modify the surface of nanocrystalline cellulose using TEMPO (2,2,6,6- Tetramethylpiperidine-1-oxyl) to enhance the dispersibility of NCC in the protein matrix and thereby increase the mechanical, thermal, and barrier properties. Control (0% NCC) and 1%, 3%, and 5% (w/w of protein) modified NCC (TM-NCC) and unmodified NCC (U-NCC) were used to fabricate films. Prepared films were characterized to evaluate film properties. XRD analysis confirmed the proper exfoliation of NCC in polymer matrix up to 3%. The tensile strength of the TM-NCC films was significantly higher than control and U-NCC films. There was no significant difference between TM-NCC and U-NCC films in terms of water vapor permeability and contact angle. However, compared to control, films with 3% and 5% TM-NCC showed significantly higher contact angles. All the films, expect 1% U-NCC, showed significantly reduced water vapor permeability compared to control. Moreover, the thermogravimetric analysis showed enhanced thermal stability due to the addition of NCC. The results demonstrated that the addition of NCC enhanced the thermal, barrier, and mechanical properties of canola films while TEMPO modification further increased the tensile properties of films compared to U-NCC films.

**Daily Development of Nutritional Composition of Canola Sprouts Followed by Solid-state Fungal Fermentation.** Ahmad Alhomodi, South Dakota State University, USA; Andrea Zavadil, South Dakota State University, USA; Mark Berhow, USDA ARS NCAUR, USA; William Gibbons, South Dakota State University, USA; Bishnu Karki, South Dakota State University, USA

The increase demand of canola oil in recent times has led to an increase in the production of defatted meal a co-product of canola oil extraction. The meal has high protein and a well-balanced amino acids profile that is desirable for industrial application. However, the meal remains underutilized due to the presence of anti-nutritional factors (ANFs) (such as glucosinolates, phytic acid and high fiber content) that lowers the digestible energy content and limit its use. Therefore, we apply sprouting as a beneficial way to increase the nutritional value of the original seed. Besides, fungal fermentation of sprouts for further improvement of sprouts composition by reducing ANFs and concentrating protein content. This study characterized the daily nutritional changes in canola sprouts and further evaluated the effect of fungal fermentation on 144h sprouts under solid state fermentation conditions. Sprouting process resulted in high moisture containing sprouts (75.3%) due to water uptake by seeds. The oil content of sprouts (27.17% at 144h) was significantly reduced when compared to raw seeds (39.57%). Likewise, phytic acid, crude fiber, acid detergent fiber, and neutral detergent fibers were reduced by 49.7%, 32.8%, 19.7%, and 16.6 % respectively, when compared to raw seeds. There was significant increase in protein and carbohydrate contents of sprouts and glucosinolates also increased from 1.27 to 3.5μM/g post sprouting. Fungal fermentation with Neurospora crassa resulted in the highest protein increase (32.84%). Heat-sterilization reduced glucosinolates by 38.8% and a further reduction (4%) was obtained by fermentation with Trichoderma reesei. A reduction in phytic acid content of 81.4%, 45.8% and 10.2% was achieved by fermentation with N. crassa, T. reesei, and Aureobasidium pullulans, respectively. Total carbohydrate was reduced by 3.3 mg/ml post heat heat-sterilization, and fungal fermentation led to the further reduction of total carbohydrates, but total fiber was found to be increased post fermentation.

**Egg White Ovomucin-derived Glycopeptides as Anti-adhesive Agents Against helicobacter Pylori Infection.** Xiaohong Sun, Qiqihar University, China (People's Republic); Songyuan Zhang, Qiqihar University, China (People's Republic); Chibuike Udenigwe, University of Ottawa, Canada

Justification: The overall prevalence of H. pylori infection is 44.3% worldwide. H pylori was classified as a class I carcinogen by World Health Organization (WHO). Antibiotics are widely used to treat H. pylori infection, which induce drug-resistant bacteria. H. pylori infection is initiated by bacterial adhesion to gastric tissues. Agents that can inhibit H. pylori attachment to gastric tissues, and thus reduce the incidence of infection, are promising alternatives to antibiotics.

Objective: The objectives of this work were to evaluate the anti-adhesive potential of ovomucin-derived glycopeptides against H. pylori and to elucidate the underlying anti-adhesive mechanism.

Methods: Ovomucin was hydrolyzed by four proteases including Protease N, Protease P, Trypsin and Pronase. The anti-adhesive activity against H. pylori were determined using the human gastric mucosal epithelial cell culture (GES-1) in vitro. The immunofluorescence experiment was applied to further validate the anti-adhesive activity of ovomucin hydrolysates. Dot-blot assay was used to reveal the possible anti-adhesive mechanism. Ovomucin-derived glycopeptides responsible for the anti-adhesive activity were purified by SDS-PAGE and western blot methods, which were further subjected to the mass spectrometry analysis.

Results: Protease N-, Pronase-, and Trypsin-ovomucin hydrolysates exhibited anti-adhesive activity at a concentration of 10 mg/mL, and the inhibitory rates were 28.7%, 25.7% and 44.8%, respectively. Overall, ovomucin hydrolysates had better anti-adhesive activity against H. pylori than that of Rebamipide (positive control, inhibitory rate was 23.1%). The possible underlying mechanism was due to ovomucin glycopeptides acting as decoys to bind with H. pylori. Ovomucin glycopeptides with glycan structures of N-acetylneuraminic acid (Hex5HexNAc4NeuAc2) might be the main binding sites for H. pylori.

Significance: This work indicated that egg white ovomucin has promising potential to be developed as anti-adhesive agents against H. pylori infection, which provided opportunities to open new windows for non-food applications of ovomucin.

**Performance of Rainbow Trout Fed Processed Carinata and Canola Meal.** Bishnu Karki, South Dakota State University, USA; Ahmad Alhomodi, South Dakota State University, USA; Tom Kasiga, South Dakota State University, USA; Michael Brown, South Dakota State University, USA; William Gibbons, South Dakota State University, USA

Aquaculture is one of the fastest growing food-producing industry. But the high feed cost of fish meal (FM) due to the limited supplies from the wild captured fisheries is the main pressing reason that plant- based proteins have been researched significantly as an alternative to FM. Proteins of plant origins are sustainable and economical, however their use is limited due to the presence of antinutritional factors (ANFs) and low digestibility and functionality. Processing of these proteins using different methods (physical, enzymatical, microbial) can reduce the level of ANFs and enhance the protein digestibility. Hence, in this study hexane extracted carinata meal (washed, enzyme treated, fungal fermented) and canola meal (raw, washed, sprouted) were tested at 30% inclusion rate in the diet of Rainbow Trout to determine the apparent digestibility coefficients (ADCs) of protein. ADCs results on carinata meal showed that mild pretreatment of carinata meal was best among all treatments with the resulting ADCs of 66.55%, which was further increased to 71.14% upon fermentation with Aspergillus niger under solid state conditions. Likewise, digestibility of washed canola was 86.09% as compared to ~81% for raw canola meal. Sprouting had positive impact on the digestibility however it was lower than the washed meal. These results indicated that mild processing of oilseed meals can enhance the nutritional quality of the oilseed meals suggesting possibility of replacing FM in feed diets with plant-based proteins.

**The Structure and Surface Activity of Proteins in Surfactants and Surfactant Mixtures.** Jason Berberich, Miami University, USA; Kayla Thompson, Miami University, USA; Kerri Peterson, Miami University, USA; Evan Danielson, Miami University, USA; Jason Boock, Miami University, USA

Mixtures of proteins and surfactants are found in many industrial applications including the processing of foods, biocatalysis in multiphase systems, and the development of pharmaceutical, cosmetic and cleaning products. Ionic surfactants are known to form complexes with proteins and perturb protein structure at low concentrations. However, many nonionic and zwitterionic surfactants interact weakly with proteins causing minimal change in structure. An understanding of the structure of protein and surfactant in mixtures is important for product formulation and development since the structure of the protein-surfactant complex can affect protein function. Changes in the structure may impact enzymatic activity, as well as surface activity, which is important for emulsion and foam formation and stability. We used a variety of biophysical characterization methods to investigate the impact of sodium dodecyl sulfate (SDS), and lauryldimethyalamine oxide (LDAO), and their mixtures, on the structure of β-lactoglobulin (βLG) and bovine serum albumin. Pyrene fluorescence showed that the presence of protein lead to the

formation surfactant aggregates at concentrations lower than the critical micelle concentration when the surfactant mixtures were composed primarily of SDS. The tertiary and secondary structure of βLG was found to be significantly perturbed by SDS and LDAO; however, much higher concentrations of the surfactant mixtures were required to unfold the protein. The equilibrium and dynamic surface tensions of the surfactants and surfactant mixtures were found to be lower in the presence of protein. The impact of the structure of the protein-surfactant complex on the equilibrium and dynamic surface tension will be discussed.

## Protein and Co-Products Division Student ePoster Pitch Competition

*Judges Jianping Wu, University of Alberta, Canada; Xiaohong Sun, Qiqihar University, China (People's Republic); Denis Chereau, IMPROVE, France*

**Bioinformatics analysis of adhesin-binding potential and ADME/Tox profile of anti-Helicobacter pylori peptides derived from wheat germ proteins.** Chi Dang, University of Ottawa, Canada; Chibuike Udenigwe, University of Ottawa, Canada; Xiaohong Sun, Qiqihar University, China (People's Republic); Ogadimma Okagu, University of Ottawa, Canada

Adhesion to host cell surface is the prerequisite step for establishing H. pylori infection. Recently, anti-adhesive activity of wheat germ-derived peptides was reported, and is considered as one of the promising strategies for preventing H. pylori infection. The underlying mechanism of action was due to peptides acting as receptor analogues and binding to H. pylori. However, molecular interaction of this binding potential and drug-likeness of the food-derived bioactive peptides are not well understood. Using bioinformatic tools, the ADME/Tox profile, together with pepsin-hydrolyzed biostability index of the peptides were analyzed to provide a shortlist of intact, non-toxic wheat germ peptides from a large database generated from a previous in vitro study. These peptides were subjected to molecular docking study using Chimera USF and Autodock Vina to help understand the molecular interactions of the potential binding between wheat germ peptides and the two most dominant H. pylori adhesins, BabA and SabA. The binding affinities (–5.9 to –7.4 kcal/mol) at lowest root-mean-square deviation (RMSD) indicated highly possible binding capabilities of the peptides to the adhesins. Docking results also helped in visualizing a multitude of different binding positions of peptides to H. pylori adhesins, that could induce conformational changes of the adhesin proteins upon binding and mitigate H. pylori infectivity. The best peptide candidates will be selected for in vitro validation, and will confirm that wheat germ-derived peptides are promising nutraceutical candidates for treatment of H. pylori infection.

**Development of Chicken Feathers Derived Novel Adsorbent for the Removal of Heavy metals from Contaminated Water.** Muhammad Zubair, University of Alberta, Canada; Aman Ullah, University of Alberta, Canada; Roopesh Syamaladevi, University of Alberta, Canada

Water is the one of the most precious resources on the planet earth. However, discharge of wastewater into the environment has increased tremendously due to industrial activities. Worldwide, heavy metal water contamination is the biggest issue, alone arsenic in groundwater has affected nearly 140 million people of the 70 countries. Through this study, we developed an eco-friendly and integrated water treatment, based on chicken feathers, with the potential to remove heavy metals without further damaging the environment. Chicken feathers have 90% of keratin proteins which were extracted using reducing agents and transformed into adsorbent by chemical treatment with graphene oxide. In this study, chicken feathers were washed, ground and then dissolved using urea and sodium sulphite solutions. After dissolution, keratin proteins were isolated from the solution using dialysis followed by freeze drying to obtain keratin proteins powder. The powdered keratin was treated with water dispersed graphene oxide and sonicated for an hour. Then, mixture was heated at 70 °C and water was removed to obtain the chicken feathers/graphene oxide adsorbent. The adsorbent was tested for heavy metal decontamination from laboratory synthetic water. FTIR analysis showed that physical interactions were developed between keratin proteins and graphene oxide. Furthermore, transmission electron microscopy displayed homogeneous mixing of graphene oxide into the protein matrix. Heavy metal adsorption experiments revealed that adsorbent can remove As, Se, Pb, Cu, Mn, Cd up to 99%. This excellent adsorption capacity was ascribed to the incorporation of graphene oxide which increased the surface affinity of the keratin molecules to adsorb heavy metals easily. In conclusion, the development of this environmentally benign process which also has lower cost and can be implemented on a large scale with an excellent removal of heavy metals from water, we can reduce the potential health and environmental impacts even before their risk of occurrence.

**Effects of Enzymatic Extraction of Oil and Protein from Chickpea Flour on Protein Functionality.** Kazunori Machida, UC Davis, USA; Fernanda Furlan Goncalves Dias, University of California, Davis, USA; Juliana Leite Nobrega de Bell, UC Davis

Aqueous (AEP) and Enzyme-Assisted Extraction Processes (EAEP) are environmentally friendly strategies that can simultaneously extract proteins, oils, and carbohydrates from food matrices. The effects of using enzyme to assist the extraction of chickpea flour were evaluated with respect to oil and protein extractability and the functional properties of the extracted protein. Different enzyme combinations were evaluated: 0.5% protease (EAEP 1), 0.5% cellulase and 0.5% protease (EAEP 2), and 0.17% cellulase, 0.17% hemicellulase, 0.17% xylanase, and 0.5% protease (EAEP 3). For EAEP 2 and EAEP 3, a pretreatment with selected carbohydrases was performed at pH 6 before the addition of the protease. The EAEP increased oil extractability from 49.8% (AEP) to 72.0—77.1% and protein extractability from 62.8% (AEP) to 83.49—86.13%. Proteins extracted from EAEP showed significant improvement in solubility (25.6% AEP proteins vs. 68.2—73.6% EAEP proteins) and digestibility (83.8% AEP proteins and 90.79—94.67% EAEP proteins) due to the use of enzyme in the EAEP. Enzymatic hydrolysis of chickpea proteins into smaller peptides was demonstrated by increased degree of hydrolysis (10% AEP and 23.3—25.5% EAEP) and decreased molecular weight of proteins/peptides in SDS-PAGE, where proteins up to 91.7 and 21.5 kDa were observed in the AEP and EAEP skim, respectively. While no significant change in oil and protein extractability was observed with the use of carbohydrases before the addition of protease, carbohydrate extraction yields improved with the pretreatment (4.3% AEP and 5.9% EAEP 1, 8% EAEP 2, and 8.3% EAEP 3). Additional use of α-galactosidase in the extracted protein (skim fractions) completely hydrolyzed flatulence-causing oligosaccharides (stachyose and raffinose) into simple sugars (glucose, fructose, and galactose). This incipient study highlights that adequate enzyme selection can lead to increased extractability of lipids and proteins and production of proteins with desired functionality.

**Examination of an efficient extraction method for rice-bran albumin and its suppressive effect on postprandial blood glucose elevation.** Rio Ogawa, Kyoritsu Women's University, Japan; Chiaki Sugimoto, Nihon University, Japan; Sunao Kotani, Nihon University, Japan; Shigenobu Ina, Nihon University, Japan; Kazumi Ninomiya, Kyoritsu Women's University, Japan; Yusuke Yamaguchi, Nihon University; Japan Hitoshi, Kumagai, Kyoritsu Women's University, Japan; Hitomi Kumagai, Nihon University, Japan

Diabetes mellitus is one of the serious diseases because it may lead to various complications. For prevention of diabetes mellitus, it is effective to suppress or retard the increase in postprandial blood glucose level. We have already shown that rice-endosperm albumin (REA) suppresses the elevation of postprandial blood glucose level and rice-bran albumin (RBA) has glucose-adsorption capacity to the same level as REA in vitro. However, there still remains a problem on poor extraction efficiency of RBA probably due to the interaction with polysaccharides such as starch and dietary fiber. In addition, suppressive effect of RBA on postprandial hyperglycaemia in vivo has not been examined yet. Therefore, in this study, we attempted to improve the extraction efficiency of RBA using polysaccharidases and examined the suppressive effect of RBA on postprandial blood glucose elevation in vivo. RBA was extracted in a citrate buffered solution using hemicellulase followed by various saccharifying enzymes and proteases. After the enzymes were inactivated by heating in boiling water, the solution was centrifuged, and the amounts of reducing sugar and protein in the obtained supernatant were measured by the Somogyi-Nelson method and the bicinchoninic acid method, respectively. Next, the suppressive effect of RBA on postprandial hyperglyceamia in vivo was evaluated by administering RBA with glucose to rats and measuring the blood glucose level. After oral administration of RBA together with glucose, the blood was collected from the tail vein over time to measure the blood glucose level. The protein concentration in the extract from rice bran increased three times by the addition of hemicellulase and digestible proteins was effective for efficient extraction of RBA. Furthermore, similar to REA, RBA delayed the elevation of postprandial blood glucose level in vivo, the peak of blood glucose and insulin levels being shifted from 15 min to 30 min. Therefore, RBA can be used as a material for functional food to prevent diabetes mellitus.

**Health Beneficial Bioactivities of Faba Bean Flour after In vitro Gastrointestinal Digestion.** Delphine Martineau-Côté, McGill University, Canada; Allaoua Achouri, Agriculture and Agri-Food Canada, Canada; Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada; Janitha Wanasundara, Agriculture and Agri-Food Canada, Canada; Salwa Karboune, McGill University, Canada

With the current environmental, public health and food security challenges, there is an increasing need for new high quality and more sustainable protein sources. Faba bean is an underutilized pulse with many favourable characteristics, including a high protein content (28–32%). This study aimed to assess the health-promoting bioactivities of faba bean proteins after gastrointestinal digestion. Three Canadian faba bean varieties (Fabelle, Malik and Snowbird) were compared to two control legumes (pea and soy). Processed legume flours (dehulled and boiled) were digested in vitro using a standardized gastrointestinal INFOGEST digestion protocol, to which a digestion phase was added to mimic brush border digestion. The digestates were filtered on a 3 kDa molecular weight cut-off membrane and peptides were recovered in the permeate to assess

their antioxidant, antidiabetic and antihypertensive activities). These bioactivities were selected based on an in silico analysis with the BIOPEP database. The results showed no significant differences between the faba bean varieties for the antioxidant bioactivity tested, except for the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, in which the Fabelle variety had a significantly ($p < 0.05$) lower activity (IC50=114±7 μg/mL) compared to Malik (IC50=89±2 μg/mL) and Snowbird (IC50=82±7 μg/mL). Overall, faba bean had either similar or better antioxidant properties (ABTS, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Iron chelating and Oxygen radical absorbance capacity (ORAC)) than pea and similar or lower antioxidant properties than soy. The antihypertensive properties (Angiotensin-converting enzyme inhibition) of the Fabelle (IC50=1348±148 μg/mL) and Malik (IC50=1497±175 μg/mL) varieties were significantly higher than pea (IC50=2222±376 μg/mL) but lower than soy (IC50=639±21 μg/mL). Similarly, the antidiabetic activity (Dipeptidyl-peptidase-IV inhibition) was higher for soy, comparatively to faba bean and pea. The results suggest that faba bean has an interesting bioactive potential against non-communicable diseases and could be valued as a health promoting food ingredient. Further confirmation of this potential in vivo is required.

**Modulating intermolecular interactions of pea protein isolate to improve its functional properties.** Yanting Shen, Kansas State University, USA; Yonghui Li, Kansas State University, USA

Proteins exist in numerous spatial arrangements that are stabilized by various intermolecular and intramolecular forces. Different denaturants such as sodium sulfite, urea, sodium dodecyl sulfate, and trypsin can interrupt protein interactions, particularly disulfide bond, hydrogen bond, hydrophobic interaction, and peptide bond, respectively, and thus altering protein secondary and tertiary structures and technological functional properties. The objectives of this study were to investigate the functional properties of pea protein isolate by modulating protein covalent and non-covalent interactions, and understand the physicochemical characteristics (e.g., free amino group, free sulfhydryl, surface hydrophobicity, SDS-PAGE, secondary structure, and SEM morphology) of the modified pea proteins that are responsible for the functional changes. The modified pea proteins were prepared by reacting the protein with sodium sulfite (0.5% and 1%), urea (0.2M and 1M), sodium dodecyl sulfate (0.1% and 0.5%), and trypsin, respectively.

Both urea and sodium dodecyl sulfate modified pea protein had significantly higher water holding capacity and oil holding capacity with up to 5.1 g H2O/g protein, and 3.1 g oil/g protein compared with the control pea protein of 4.1 g H2O/g and 1.3 g oil/g, respectively. Sodium sulfite modified protein also exhibited higher oil holding capacity of up to 2.8 g oil/g. There was no significant difference for the emulsifying properties of the modified proteins. All the denaturation approaches significantly increased protein solubility up to 80 % compared with the control pea protein of 47 % at pH 7. The pea protein hydrolyzed by trypsin possessed the highest solubility (48%) compared with the control (15%) at the isoelectric point (pH 4.5). It also exhibited the highest foaming capacity and better gelation properties than the other modified proteins. This study provides a fundamental knowledge of protein functionalities as related to protein covalent and non-covalent interactions, and will contribute to broader food applications of pea protein.

## Protein-based Hydrocolloids for Food and Biomaterial Applications

*Chairs Lingyun Chen, University of Alberta, USA; Dario Cabezas, UNQ—CONICET, Argentina*

**A Facile Method to Fabricate Size-controllable Whey Protein-based Microgels and Their Application as Oil-in-water Emulsifiers.** Yifu Chu, University of Alberta; Canada; Yeonji Jo, University of Alberta, Canada; Lingyun Chen, University of Alberta, USA

Protein microgels are increasingly being viewed as promising building blocks for diverse food applications, such as for fat replacement and formulation of high-protein beverages. This study described a facile method to fabricate whey protein microgels of controllable size by modulating the protein-polysaccharide interactions. The spherical microgels were monodispersed and their size could be precisely controlled to 1, 3 and 6 μm. We also explored the potential of these protein microgels to act as oil-in-water emulsifiers, and the results demonstrated that the presence of the microgels significantly improved the emulsion stability over 28 days of ambient storage. The emulsions (30% w/w oil) maintained extremely high viscosity at very low microgel concentration (1%) and efficiently stopped oil droplets from flocculation and coalescence during storage. This might open a route to modulate the bulk viscosity by adding the protein microgels as fat replacers in certain food products.

**Construction of Robust Assembled Prolamin Protein Hydrogels with Double-crosslinked Networks.** Yixiang Wang, McGill University, Canada; Jing Jing Wang, Foshan University, China (People's Republic); Song-Qing Hu, South China University of Technology, China (People's Republic); Lingyun Chen, University of Alberta, USA

Hydrogels are a unique form of soft and wet elastomers with a three-dimensional hydrophilic network, entrapping a large amount of water while maintaining their network

structure integrity. However, the traditional hydrogels are vulnerable to stress-induced deformation and propagation of cracks, which may lead to a dangerous loss in load-bearing capacity and limit their practical uses. This study introduced uniquely constructed double-crosslinked hordein/zein hydrogels with outstanding mechanical properties. Notably, the optimized hydrogels demonstrated a compressive stress of 1.90 MPa at a strain of 70%, excellent self-recovery after 40 cycles of loading-unloading treatments, and superior foldable properties. Further study of the hydrogel nanostructures and properties revealed that hordein highly participated in the formation of chemically cross-linked networks that maintained the elasticity of the hydrogels; whereas physical cross-linked domains that consisted of beadlike particles by hordein/zein assembly were evenly integrated inside the large chemical crosslinked framework and acted as ''load carriers'' to effectually absorb energy. Consequently, the intertwined spatial network structures and beadlike particles collectively and efficiently dispersed and absorbed energy to withstand large deformations throughout the chemically and physically crosslinked networks. Such a prolamin protein-based hydrogel has potential to be used in bio-based load-bearing soft devices, which will diversify the use of zein and hordein as the by-products of maize and barley. In addition, the generated knowledge may offer new opportunities to design and construct strong hydrogels from many other plant protein resources to unlock their potential as biopolymer and biocompatible materials.

**Effect of Ultrasound on Compositional and Emulsifying Properties of Soybean Okara.** Dario Cabezas, UNQ - CONICET, Argentina; Yeisson Moscoso Ospina, UNQ - CONICET, Argentina; María Cecilia Porfiri, UNQ - CONICET, Argentina

High-intensity ultrasound is widely used in the food industry to modify structural and functional properties of macromolecules (proteins, polysaccharides, etc.), and to expand the range of potential applications. The objective of this research was to understand the effects of different ultrasound treatments on the compositional and emulsifying properties of different samples of soybean okara. Defatted solvent-free soy flour was extracted by adding 11 times the weight of distilled water, adjusting the dispersion to pH 9.0, and gently stirring during 30 min at 60 $\pm$ 2°C. The insoluble residue obtained after centrifugation (7000 g, 4°C, 15 min) was dried by a treatment with 2-propanol. This process allowed to obtain the UOK sample (un-sonicated okara). Furthermore, the soybean flour dispersion was subject to treatment in a probe-type ultrasonic homogenizer (Sonics Vibra Cells- 7070 J, 75% power, 10 min) before or after the alkaline extraction process, thus allowing to obtain the SOK and OKS samples, respectively. SOK have a higher yield and protein content than UOK and OKS, due to the insolubilization of protein structures after the ultrasound pretreatment. This compositional characteristic is caused by an increase in the content of the glycinin (11S) fraction of soybean storage proteins observed in its electrophoretic profile. In this sense, SOK dispersions presented the highest increase in the interfacial pressure at equilibrium (TD3 LMT-850, LAUDA) and in the complex viscosity at the oil/water interface as a function of time (AR-G2, TA Instruments). These characteristics allowed this sample to present the best emulsifying properties among the evaluated samples, analyzing the variation of De Brouckere (D4,3) mean diameters and the percentage of serum layer (SL%) as a function of time of coarse emulsions for 1 hour. In conclusion, the sonication process allows to generate compositional and structural modifications in the okara soybeans, thus varying their emulsifying properties significantly.

**Food Hydrocolloids: Application as Functional Ingredients to Control Lipid Digestion and Bioavailability.** Hualu Zhou, University of Massachusetts Amherst, USA; David McClements, University of Massachusetts Amherst, USA

One of the most popular research areas in food hydrocolloids over the past decade or so has been their application as functional ingredients to modulate the gastrointestinal fate of foods. In particular, they are being utilized to control the hydrolysis of macronutrients (such as fat, protein, and starch) in the gastrointestinal tract, as well as to alter the pharmacokinetics and bioavailability of hydrophobic bioactive agents, including oil-soluble vitamins (e.g., vitamin A, D, E, and K), nutraceuticals (e.g., carotenoids, phytosterols, curcumin, resveratrol, and quercetin) and healthy lipids (e.g., omega-3 fatty acids and conjugated linoleic acids). Food hydrocolloids may be naturally present in foods (such as fruits, vegetables, seeds, and cereals), they may be added as functional ingredients (such as thickening, gelling, emulsifying or stabilizing agents), or used to construct colloidal delivery systems (such as emulsions or microgels). Hydrocolloids can be used to protect bioactive agents from chemical degradation within foods and beverages during storage, but then increase their bioavailability after consumption. Moreover, they can be used to target the delivery of bioactive agents to particular sites in the gastrointestinal tract or to modulate their release profile. Food hydrocolloids are therefore versatile natural ingredients for the formulation of a new generation of functional food products designed to enhance human health and wellbeing. This article provides a review of the application of food hydrocolloids, mainly proteins and polysaccharides, for modulating the gastrointestinal fate of functional foods, with an emphasis on their ability to control macronutrient digestion and bioactive bioavailability.

**Interactions of Food Colloids During Gastric Digestion: Implications for Nutrient Delivery and Absorption.** Harjinder Singh, Massey University, New Zealand
Our foods are contained within complex structures/ matrices, created through self-assembly and interactions of macromolecules. During the last decade, a growing body of evidence has shown that food structures play a key role in the kinetics of transit and digestion and absorption of nutrients. Gastric digestion is a crucial step where the food structures are extensively modified, due to mixing with the digestive juices (containing various minerals, and enzymes) at a highly acidic pH. There is also mechanical agitation due to peristalsis in the stomach. The food breakdown process and the resulting modified food structures play a key role in gastric emptying and consequently the delivery of nutrients in the duodenum. In this context, it is possible to design food structures that exert specific biophysical behaviour in the stomach modulating postprandial physiological responses. For instance, liquid milk can self-structure to form a curd inside the stomach, through the action of pepsin and acid on the casein micelles, influencing the stomach emptying rates of both protein and lipids. Emulsions systems that become unstable or remain stable or form gels in the stomach can be designed to influence rate of delivery of lipids to the small intestine. However, the fundamental mechanisms involved in gastric emptying, breakdown and mixing kinetics of different food materials in in the gastric environment need to be better understood. The knowledge about digestive behaviour of food materials will allow the food industry to develop the next generation of health-enhancing foods.
This presentation will provide an overview of the specific protein-based colloidal structures in selected dairy and plant foods and will highlight recent studies on modification of these structures during digestion. Particular emphasis will be placed on impact of these structural changes on the rates delivery of protein and lipid to the small intestine.

**Protein-based Polymer Materials for Agricultural and Biomedical Applications.** Alberto Romero, Universidad De Sevilla, Spain; Mercedes Jiménez-Rosado, Universidad de Sevilla, Spain; Victor Perez-Puyana, Universidad de Sevilla, Spain; Antonio Guerrero, Universidad de Sevilla, Spain
Proteins display an essential role in numerous natural systems due to their structural and biological properties. Given their unique properties, proteins have been thoroughly investigated in the last few decades, offering a myriad of solutions for the development of innovative protein-based materials within the sustainable polymers context. In this context, proteins can be obtained from waste and by-products from other industries (mainly agri-food), which lowers their costs and allows them to make the most of the available resources, contributing to support the circular economy. In the agricultural and horticultural sectors, protein-based bioplastics can be used for the controlled release of nutrients, modulated with their biodegradability and can absorb and retain a large amount of water, supplying it to crops in drought times. Thus, the efficiency of their assimilation by crops is improved, avoiding the excessive use of fertilizers. On the other hand, protein sources can develop biomaterials, including films, foams, composites and gels, particularly for biomedical applications such as drug delivery systems, biosensors and, especially, scaffolds for tissue regeneration. Biomaterials must fulfil specific requirements such as specific geometry and microstructure, enough mechanical integrity and, fundamentally, biocompatibility, which makes proteins excellent candidates. In any case, the development of protein-based materials requires paying great attention to the raw materials used, passing through production methodologies of raw materials, and the processing methods of final materials for specific applications in order to reach the required functional characteristics. In this sense, current and future trends of the development of protein-based materials for agricultural and biomedical applications are reported and compared.

**Research Investment Agenda Plant-Based Foods & Proteins 2030: Developing The Road Map.** Presenting Author: Gerard Klein Essink, Bridge2Food, Netherlands; Chair and Moderator Scott, Bloomer, American Oil Chemists' Society, USA
The Aim of the "Global Plant-Based Foods Eco-System (Eating, Living, Lifestyle)" is to accelerate the shift towards a more plant-based diet, with better foods, produced in is a sustainable way, as well as increasing food security with an adequate supply of plant protein crops & proteins: Plant-based foods require sustainable and nutritionally-sound ingredients, meal components and meal solutions which enable citizens to shift and stick to a plant-based diet (a diet based on products from plants, excluding animal-based products like meat, fish, dairy, eggs and insects and including algae/ fungi/ microorganisms-based products).

## Updates on Protein Research in China

*Chairs Xiaonan Sui, Northeast Agricultural University, China (People's Republic); Keshun Liu, U.S. Department of Agriculture, USA*

**Changes in Protein Quality, Fat Content and Structural Properties During Seed Germination of Flaxseed (Linum Usitatissimum L.)** Xiuzhu Yu, Northwest A&F University, China (People's Republic)
Flaxseed (Linum usitatissimum L.) has garnered a great deal of research attentions in recent years as a

highly nutritious food material. This study characterized the changes of crude protein, lipids, free amino acids, amino acid composition, structural properties of flaxseed during a 7-day germination period by various chemical and chromatographic methods. The results showed that germination for one day brought about a 1.29-fold increase in Cys and 1.39-fold increase in Tyr as compared to ungerminated flaxseed, while the triolein content slightly increased. Some high molecular proteins were hydrolyzed into smaller proteins, peptides and amino acids after germination. Scanning electron micrographs (SEM) showed that the germination process destroyed the interactions among protein, lipid, and cellulose and exposed many independent fiber structures. X-ray diffraction analysis showed that germination did not change the amorphous structure of flaxseed. The results of present study suggest that germination effectively enhance the nutritional value and alter the physicochemical characteristics of flaxseed.

**Evolution of the Interaction Between Polyphenols and Proteins: From Disordered Aggregates to Ordered Supermolecules for Precise Nutrition Based on Targeting Gut Microbiota.** Bing Hu, Nanjing Agricultural University, China (People's Republic)
"Interaction between polyphenols and proteins is a very hot topic, which are very important for the food property, quality and nutrition. It is well known that the interaction between polyphenols and proteins, even at very low concentrations results in the aggregates and precipitates. We demonstrate for the first time that polyphenols attach to the surface of food protein amyloid fibrils via hydrogen bonding, π-π stacking, and hydrophobic interactions, followed by the deposition and self-assembly of the polyphenol molecules into hybrid nanofilaments and functional macroscopic hydrogels made thereof. A key finding which emerges from this work is that Polyphenols not only inhibit protein amyloid fibril formation or remodel mature amyloid fibrils, as already reported at low protein concentrations, but can also accumulate and grow on amyloid fibril surface. It also represents an excellent approach for food molecular processing, to create "future food" without addition of salt, sugar, or other chemical additives. These hybrids reinforce the properties and functionalities of single polyphenols, specifically by allowing the use of high concentrations with chemical stability, which would not be possible with polyphenols alone: the levels of polyphenols in the hydrogels are as high as 4.0 wt%, which, to the best of our knowledge, is the highest polyphenol loading level among delivery systems ever reported in the literature. Most importantly, amyloid fibrils and polyphenols act as not only the building blocks but also functional materials with anti-bacterial and anti-inflammatory activities. This is a new finding which may have groundbreaking consequences in the treatment of chronic gastrointestinal diseases by "drugging the microbiome" with natural bioactive ingredient systems, especially for blocking the expansion of bacteria driven by intestinal inflammation.

**Fabrication of Core-shell Nanofibers by Electrospinning of Gelatin-based Emulsions.** Hui Zhang, Zhejiang University, China (People's Republic)
The aim of the work was to fabricate core-shell nanofibers electrospun from corn oil-in-water (O/W) gelatin-based emulsions for food applications of bioactive encapsulation and controlled release. The fiber morphology and microstructure of electrospun nanofibers were analyzed by scanning electron microscopy (SEM), transmission electron microscope (TEM), and confocal laser scanning microscopy (CLSM). The thermal behavior and possible interactions of nanofiber mats were evaluated using thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR). The surface hydrophobicity and mechanical properties were evaluated by water contact angle and mechanical measurements. Additionally, the rheology and microstructure of the gelatin-stabilized emulsions were characterized by rheological measurements and optical microscopy. Results showed that the emulsions showed shear-thinning and predominantly elastic gel behaviors. Core-shell nanofibers were obtained by electrospinning of gelatin-stabilized emulsions. The encapsulated oil was randomly distributed as core, especially inside the beads. The electrospun fibers showed good storage stability and thermal decomposition stability. Furthermore, core-shell nanofibers were electrospun from emulsions stabilized by the bilayer of gelatin-gum Arabic complexes. The bilayer method could enhance the emulsion stability by progressively increasing the viscosity of emulsions, which contributed to the formation of bead-free and smooth electrospun fibers with the increasing diameters. To improve the mechanical and surface hydrophobic properties of electrospun fibers, genipin was investigated as a safe cross-linking agent for gelatin-based electrospun fibers. These results contributed to a good understanding on the emulsion electrospinning of food materials for potential food applications.

**Overview of Plant-based Food Market and Consumers in China.** Chichi Hong, Heymaet Food Technology Co., Ltd., China (People's Republic)
China is the biggest meat consumption market in the world. Many alternative meat companies are exploring the possibilities of entering the market. Who are the targeted consumers? What kind of products would be popular? What are the channels for the Chinese consumers to purchase the products? What are the differences between the Chinese and the US market? Here you can get some thinking from a Chinese plant-based meat start-up.

**Plant Protein Combined with Complementary Protein or Fiber for a Sustainable and Healthy Future.** Yan Zheng, Wilmar Wilmar International, China (People's Republic) Xuebing Xu, Wilmar International, China (People's Republic)
The awareness about the challenge of environmental constraints and an ever-growing world population, also the increasing health conscious promote the search for more sustainable, high-nutritional and environmental-friendly foods, especially the exploration of alternative protein sources. In this report, three aspects will be discussed. Firstly, as consumers are demanding an extra level of sophistication regarding to plant-based diet and products, complementary protein blends should be pay more attention, since protein blends from various origins may have preferential benefits like balanced amino acid composition, combined physiological activity, preferred and positive consumer labeling. Secondly, the combined health and functionality benefits, as well as the challenge of protein/fiber complex will be discussed. The protein/fiber complex could be served as novel plant-based ingredient, which ensure the feasibility of incorporating protein and fiber functionality and nutrition into market products synergistically. Lastly, the production technics of plant protein will be briefly reviewed, as the functionality and nutrition of plant protein should be greatly influenced by different manufacturing process. Moreover, from a long-term perspective the environmental impact could be hugely different.

**Soy Protein: Recent Advances in Processing Technologies.** Lianzhou Jiang, Northeast Agricultural University, China (People's Republic)
Rising health concerns and increasing obesity levels in human society have led some consumers to cut back on animal protein consumption and switch to plant-based proteins as an alternative. Soy protein is a versatile protein supplement and contains well-balanced amino acids, making it comparable to animal proteins. With sufficient processing and modification, the quality of soy protein can be improved above that of animal proteins, if desired. The modern food industry is undergoing a dynamic change, with advanced processing technologies that can produce a multitude of foods and ingredients with functional properties from soy proteins, providing consumers with a wide variety of food products. This presentation highlights recent progress in soy protein processing technologies.

**The Development History and Recent Updates on Soy Protein-based Meat Alternatives.** Xiaonan Sui, Northeast Agricultural University, China (People's Republic)
Owing to the rapid growth of the world's population and the consequent effects on the consumption of natural resources, we are facing increasing shortages in availability of proteins with high nutritional values. Additionally, considerations relating to animal welfare and human health have promoted the development of plant protein meat alternatives. The market for plant-based meat alternatives is expanding rapidly to cater to growing consumer demand. Soy protein has been successfully utilized in the preparation of meat alternatives, due to its excellent gelation properties and potential to form fibrous structures. It has become the most widely known alternative to animal proteins. High moisture extrusion is a relatively mature technology and is widely used for manufacturing soy protein meat alternatives with a similar fiber texture to meat. Further research needs to focus on optimizing technical parameters, improving nutrition and safety, and enriching product taste to meet consumer demands for product quality.

## Protein and Co-Products Poster Session

*Poster Chairs Milagros Hojilla-Evangelista, USDA ARS NCAUR Plant Polymer Research Unit, USA; Yixiang Wang, McGill University, Canada*

**Bioinformatics analysis of adhesin-binding potential and ADME/Tox profile of anti-Helicobacter pylori peptides derived from wheat germ proteins.** Chi Dang, University of Ottawa, Canada; Chibuike Udenigwe, University of Ottawa, Canada; Xiaohong Sun, Qiqihar University, China (People's Republic)
Adhesion to host cell surface is the prerequisite step for establishing H. pylori infection. Recently, anti-adhesive activity of wheat germ-derived peptides was reported, and is considered as one of the promising strategies for preventing H. pylori infection. The underlying mechanism of action was due to peptides acting as receptor analogues and binding to H. pylori. However, molecular interaction of this binding potential and drug-likeness of the food-derived bioactive peptides are not well understood. Using bioinformatic tools, the ADME/Tox profile, together with pepsin-hydrolyzed biostability index of the peptides were analyzed to provide a shortlist of intact, non-toxic wheat germ peptides from a large database generated from a previous in vitro study. These peptides were subjected to molecular docking study using Chimera USF and Autodock Vina to help understand the molecular interactions of the potential binding between wheat germ peptides and the two most dominant H. pylori adhesins, BabA and SabA. The binding affinities (–5.9 to –7.4 kcal/mol) at lowest root-mean-square deviation (RMSD) indicated highly possible binding capabilities of the peptides to the adhesins. Docking results also helped in visualizing a multitude of different binding positions of peptides to H. pylori adhesins, that could induce conformational changes of the adhesin proteins upon binding and mitigate H. pylori

infectivity. The best peptide candidates will be selected for in vitro validation, and will confirm that wheat germ-derived peptides are promising nutraceutical candidates for treatment of H. pylori infection.

**Characterization of walnut flour by-product of oil extraction and its use in a gluten-free sponge cake.** Dario Cabezas, UNQ - CONICET, Argentina; Juan J Burbano, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (Facultad de Cs Exactas-UNLP, CIC, CONICET), Argentina; María Jimena Correa, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (Facultad de Cs Exactas-UNLP, CIC, CONICET), Argentina

Walnut flour (WF) is a by-product of the edible oil industry, obtained from the press cake milling. The compositional characteristics (high lipid, protein, and fiber contents) made WF attractive for improving the nutritional quality of gluten-free products. This work aimed to characterize WF and evaluate the effect of WF addition on the thermal and textural behavior of batters and sponge cake quality. The proximate composition of WF and fatty acid and amino acid profiles were obtained. The control sponge cake contained rice flour and corn and cassava starches. Two levels of WF were used: 10% (WF10) and 20% (WF20). The thermal transitions and texture of batters were determined by DSC and back-extrusion, respectively. Finally, baking quality was evaluated based on specific volume and crumb hardness. WF has high lipid (55.7%), protein (24.6%), and dietary fiber content (9.4%). The primary fatty acids were linolenic acid (59.8%), oleic acid (15.47%), and α-linolenic acid (14.68). WF contains relatively high Arginine levels and a low Lysine/Arginine ratio (0.345), which has been related to an antiatherogenic effect.

During heating, the batters exhibited two endotherms corresponding to the starch gelatinization and the dissociation of the amylose lipid complex. In all formulations, gelatinization showed a split endotherm, typical of processes with water restriction. The gelatinization enthalpy decreased with increasing WF concentration, with values ranging from 6.19 J/g (control) to 3.37 J/g (WF20). The dissociation of the amylose-lipid complex showed a displacement to lower temperatures and an increase in enthalpy with WF addition. The textural parameters such as firmness and consistency index increased with WF level. Sponge cakes with WF showed higher specific volume and slightly lower crumb hardness than the control. Results show that the addition of WF affects the thermal and textural properties of batters, while also improving the baking quality of the sponge cakes.

**Comparison of ISO method 14902:2001 with new AOCS method Ba 12a-2020 for assaying trypsin inhibitor activity.** Keshun Liu, U.S. Department of Agriculture, USA; Mike Woolman, US Dept. of Agriculture, USA

For measuring trypsin inhibitor activity (TIA) of plant protein products, a new AOCS method, Ba 12a-2020, was recently approved. The method features 5 mL assay volume, the enzyme-last sequence and standardization of results against a reference trypsin with a fixed specific activity (SA) when expressed as mg trypsin inhibited (TId)/g sample. There is another official method, International Organization for Standardization (ISO) Method 14902:2001. The two methods differ in sample preparation, extraction, assay conditions and result calculation. This study was conducted to compare the two methods, using three sets of samples: assorted protein products of soybeans, beans and grains, boiled soybeans with varied duration, and toasted soy flakes with varied duration. Results show that with all the samples having varied TIA, values in mg TId/g sample measured by ISO and AOCS methods were highly correlated ($R2 = 0.9973$), giving a linear equation of $y = 0.5464x - 0.4887$, where y represents ISO values and x for AOCS values. When the ratio of ISO/AOCS in TIA values was plotted against AOCS values, the line remained relatively flat around 0.53 but started to curve down when TIA values approached the lowest. The negative y-intercept for the linear equation explains the ratio trend observed. In another experiment, raw soybeans were measured with four trypsin reagents having varied SA, respectively. Results show that for a given sample ISO values decreased with increasing trypsin SA, but AOCS values remained consistent (due to standardization against the reference trypsin). The ratio of ISO/AOCS decreased also. It is concluded that accurate and direct comparison was impossible, but for most samples ISO values were roughly 50 to 55 % of AOCS values.

**Development of Chicken Feathers Derived Novel Adsorbent for the Removal of Heavy metals from Contaminated Water.** Muhammad Zubair, University of Alberta, Canada; Aman Ullah, University of Alberta, Canada; Roopesh Syamaladevi, University of Alberta, Canada

Water is the one of the most precious resources on the planet earth. However, discharge of wastewater into the environment has increased tremendously due to industrial activities. Worldwide, heavy metal water contamination is the biggest issue, alone arsenic in groundwater has affected nearly 140 million people of the 70 countries. Through this study, we developed an eco-friendly and integrated water treatment, based on chicken feathers, with the potential to remove heavy metals without further damaging the environment. Chicken feathers have 90% of keratin proteins which were extracted using reducing agents and transformed into adsorbent by chemical treatment with graphene oxide. In this study, chicken feathers were washed, ground and then dissolved using urea and sodium sulphite solutions. After dissolution, keratin proteins were

isolated from the solution using dialysis followed by freeze drying to obtain keratin proteins powder. The powdered keratin was treated with water dispersed graphene oxide and sonicated for an hour. Then, mixture was heated at 70 °C and water was removed to obtain the chicken feathers/graphene oxide adsorbent. The adsorbent was tested for heavy metal decontamination from laboratory synthetic water. FTIR analysis showed that physical interactions were developed between keratin proteins and graphene oxide. Furthermore, transmission electron microscopy displayed homogeneous mixing of graphene oxide into the protein matrix. Heavy metal adsorption experiments revealed that adsorbent can remove As, Se, Pb, Cu, Mn, Cd upto 99%. This excellent adsorption capacity was ascribed to the incorporation of graphene oxide which increased the surface affinity of the keratin molecules to adsorb heavy metals easily. In conclusion, the development of this environmentally benign process which also has lower cost and can be implemented on a large scale with an excellent removal of heavy metals from water, we can reduce the potential health and environmental impacts even before their risk of occurrence.

**Effect of glycation on the interaction between BSA and curcumin.** Renata Pfeilsticker Neves, University of Ottawa, Canada; Ogadimma Okagu, University of Ottawa, Canada; Xiaohong Sun, Qiqihar University, China (People's Republic); Chibuike Udenigwe, University of Ottawa, Canada

It is known that glycation can improve many protein properties. However, little attention has been given to the effect of glycation on the interaction between proteins and hydrophobic compounds. The objective of this study was to determine the effect of glycation on the interactions between bovine serum albumin and curcumin.

The BSA-glucose (1:1000 molar ratio) reaction occurred at 60oC using the wet heating method. DLS was performed to obtain surface charge and size distribution of particles. The interaction of the conjugate with curcumin was studied through fluorescence quenching. The change in BSA's surface can be observed through zeta potential analysis. The surface charge decreases from –3.75 mV (native BSA) to –16.87 mV after glycation. The result is coherent given likely interaction between glucose and positively charged residues such as lysine. An increase in mean diameter can be observed for the control due to protein unfolding. Likewise, the glycated sample (gly-BSA) shows an increase in size because of the interactions with glucose.

Glucose-BSA conjugate shows a 17% decrease in surface hydrophobicity compared to the control. It is possible that the carbohydrate conjugation limits access to BSA's hydrophobic sites, perhaps sterically. Fluorescence quenching corroborates to this hypothesis as the binding affinity (K) decreased. Nonetheless, glycation seems to occur predominantly on the surface of the protein since the number of interactions in the hydrophobic core (f), and the number of curcumin molecules bound (n) did not suffer substantial discrepancy.

Glycation improves proteins' properties without compromising interactions with curcumin. Conjugate has potential in drug delivery of hydrophobic bioactive molecules.

**Effect of Storage Conditions on the Proximate Profile and Functional Properties of Whole Yellow Pea Flour.** Serap Vatansever, South Dakota State University, USA; Sushmita Karki, South Dakota State University, USA; Atanu Biswas, USDA-ARS, USA; Clifford Hall, South Dakota State University, USA

Pulse ingredients have attracted significant attention as important sources of protein and carbohydrate due to their good techno-functional properties in food applications. However, limited knowledge exists on the functional characteristics and nutrient composition of pulses that have been stored under diverse environments. The goal of this study was to assess the impacts of different storage conditions on the functional properties, associating with protein and starch, and nutrient profile. Whole yellow peas were stored 180-day under various temperatures (21 to 50 oC) and relative humidities (RH) (40 to 84%). Peas were collected at 0, 30, 60, 90 and 180 days. The samples were milled through a 0.5 mm screen and flours were analyzed for chemical composition and functional properties (i.e., emulsion and foaming characteristics, water activity index (WAI), water solubility index (WSI), pasting properties, and gel strength). The outcomes of this study showed that moisture content increased with increasing storage RH. Peas stored at higher RH with longer period had lower protein and starch contents compared to control (45% starch and 19% protein, as is). A combination of 50 oC and 84% RH at 180-day significantly ($p < 0.05$) decreased foaming capacity (235 to 50%) and stability (93 to 79%). This storage combination eliminated the potential of the flour to form an emulsion but caused high WAI and low WSI. Higher temperature with increasing RH and longer storage led to an increase in peak viscosity, a reduction in setback and cold paste viscosity and low gel strength (e.g., 16 g for flour of 180 day, 50oC, 84%RH vs. 300 g for control). Overall, changes occurring in functionalities support changes in protein and starch structure or composition. The findings of this study support that storage conditions of the pea can dramatically impact composition and functionality that are important to the food industry.

**Effects of Enzymatic Extraction of Oil and Protein from Chickpea Flour on Protein Functionality.** Kazunori Machida, UC Davis, USA; Fernanda Furlan Goncalves Dias, University of California, Davis, USA; Juliana Leite Nobrega de Bell, UC Davis, USA

Aqueous (AEP) and Enzyme-Assisted Extraction Processes (EAEP) are environmentally friendly strategies that can simultaneously extract proteins, oils, and carbohydrates from food matrices. The effects of using enzyme to assist the extraction of chickpea flour were evaluated with respect to oil and protein extractability and the functional properties of the extracted protein. Different enzyme combinations were evaluated: 0.5% protease (EAEP 1), 0.5% cellulase and 0.5% protease (EAEP 2), and 0.17% cellulase, 0.17% hemicellulase, 0.17% xylanase, and 0.5% protease (EAEP 3). For EAEP 2 and EAEP 3, a pretreatment with selected carbohydrases was performed at pH 6 before the addition of the protease. The EAEP increased oil extractability from 49.8% (AEP) to 72.0—77.1% and protein extractability from 62.8% (AEP) to 83.49—86.13%. Proteins extracted from EAEP showed significant improvement in solubility (25.6% AEP proteins vs. 68.2—73.6% EAEP proteins) and digestibility (83.8% AEP proteins and 90.79—94.67% EAEP proteins) due to the use of enzyme in the EAEP. Enzymatic hydrolysis of chickpea proteins into smaller peptides was demonstrated by increased degree of hydrolysis (10% AEP and 23.3—25.5% EAEP) and decreased molecular weight of proteins/peptides in SDS-PAGE, where proteins up to 91.7 and 21.5 kDa were observed in the AEP and EAEP skim, respectively. While no significant change in oil and protein extractability was observed with the use of carbohydrases before the addition of protease, carbohydrate extraction yields improved with the pretreatment (4.3% AEP and 5.9% EAEP 1, 8% EAEP 2, and 8.3% EAEP 3). Additional use of α-galactosidase in the extracted protein (skim fractions) completely hydrolyzed flatulence-causing oligosaccharides (stachyose and raffinose) into simple sugars (glucose, fructose, and galactose). This incipient study highlights that adequate enzyme selection can lead to increased extractability of lipids and proteins and production of proteins with desired functionality.

**Effects of pH on techno-functionality and interfacial activity of potato protein extract (PPE).** Ali Jafarpour, Danmarks Tekniske Universitet, Denmark; Egon B. Hansen, Technical University of Denmark, Denmark; Charlotte Jacobsen, Technical University of Denmark, Denmark

The discharged wastewater from potato starch factories known as potato fruit juice (PFJ) contains 1–1.5% protein with potential techno-functionalities if recovered by non-destructive techniques. In this study, the intact potato protein extract (PPE) in aqueous solution (0.5% w/v) at different pH values (2, 4, 6, 8 and 10) was characterized with respect to solubility, emulsifying activity (EAI) and stability (ESI), droplet size, zeta potential and interfacial activity of stabilized interface. In a protein solubility graph, a valley shape was observed indicating the lowest solubility around pH 6, while the highest solubility was recorded at pH 2 and pH 8 as 53.4% and 42.0%, respectively. A pH-dependent trend was observed as both EAI and ESI were increased by shifting the pH from acidic region to the alkaline with highest values at pH 10. Apparently, the negatively charged protein at the alkaline region have higher emulsifying activity compared to the positively charged protein at acidic pH. Similarly, zeta potential data was dominated by the applied pH, i.e., a remarkable shift was recorded from pH 2 (–6.1), toward pH 8 (–30.3) and 10 (–31.5), corresponding to the droplet size (D3,2) of 2810 nm, 1013 nm and 947 nm, respectively. In terms of interfacial activity, the aqueous PPE solution actively reduced the interfacial tension (IFT) of stabilized interface in water-in-oil system at different pH values. However, apart from a stronger and much faster decrease of the IFT at the alkaline region, the formed droplet detached from the needle after nearly two min and less than one min at pH 8 and 10, respectively. This phenomenon can possibly be attributed to the synergistic effect of high protein concentration of aqueous phase in our study and pH-dependency of interfacial activity of PPE with very high mobility and flexibility at alkaline pH, yet to be clarified.

**Examination of an efficient extraction method for rice-bran albumin and its suppressive effect on postprandial blood glucose elevation.** Rio Ogawa, Kyoritsu Women's University, Japan; Chiaki Sugimoto, Nihon University, Japan; Sunao Kotani, Nihon University, Japan; Shigenobu Ina, Nihon university, Japan; Kazumi Ninomiya, Kyoritsu Women's University, Japan; Yusuke Yamaguchi, Nihon University, Japan; Hitoshi Kumagai, Kyoritsu Women's University, Japan; Hitomi Kumagai, Nihon University, Japan

Diabetes mellitus is one of the serious diseases because it may lead to various complications. For prevention of diabetes mellitus, it is effective to suppress or retard the increase in postprandial blood glucose level. We have already shown that rice-endosperm albumin (REA) suppresses the elevation of postprandial blood glucose level and rice-bran albumin (RBA) has glucose-adsorption capacity to the same level as REA in vitro. However, there still remains a problem on poor extraction efficiency of RBA probably due to the interaction with polysaccharides such as starch and dietary fiber. In addition, suppressive effect of RBA on postprandial hyperglycaemia in vivo has not been examined yet. Therefore, in this study, we attempted to improve the extraction efficiency of RBA using polysaccharidases and examined the suppressive effect of RBA on postprandial blood glucose elevation in vivo. RBA was extracted in a citrate buffered solution using hemicellulase followed by various saccharifying enzymes and proteases. After the enzymes were inactivated by heating in boiling water, the solution was centrifuged, and the amounts of reducing sugar and

protein in the obtained supernatant were measured by the Somogyi-Nelson method and the bicinchoninic acid method, respectively. Next, the suppressive effect of RBA on postprandial hyperglyceamia in vivo was evaluated by administering RBA with glucose to rats and measuring the blood glucose level. After oral administration of RBA together with glucose, the blood was collected from the tail vein over time to measure the blood glucose level. The protein concentration in the extract from rice bran increased three times by the addition of hemicellulase and digestible proteins was effective for efficient extraction of RBA. Furthermore, similar to REA, RBA delayed the elevation of postprandial blood glucose level in vivo, the peak of blood glucose and insulin levels being shifted from 15 min to 30 min. Therefore, RBA can be used as a material for functional food to prevent diabetes mellitus.

**Health Beneficial Bioactivities of Faba Bean Flour after In vitro Gastrointestinal Digestion.** Delphine Martineau-Côté, McGill University, Canada; Allaoua Achouri, Agriculture and Agri-Food Canada, Canada; Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada; Janitha Wanasundara, Agriculture and Agri-Food Canada, Canada; Salwa Karboune, McGill University, Canada

With the current environmental, public health and food security challenges, there is an increasing need for new high quality and more sustainable protein sources. Faba bean is an underutilized pulse with many favourable characteristics, including a high protein content (28–32%). This study aimed to assess the health-promoting bioactivities of faba bean proteins after gastrointestinal digestion. Three Canadian faba bean varieties (Fabelle, Malik and Snowbird) were compared to two control legumes (pea and soy). Processed legume flours (dehulled and boiled) were digested in vitro using a standardized gastrointestinal INFOGEST digestion protocol, to which a digestion phase was added to mimic brush border digestion. The digestates were filtered on a 3 kDa molecular weight cut-off membrane and peptides were recovered in the permeate to assess their antioxidant, antidiabetic and antihypertensive activities). These bioactivities were selected based on an in silico analysis with the BIOPEP database. The results showed no significant differences between the faba bean varieties for the antioxidant bioactivity tested, except for the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, in which the Fabelle variety had a significantly ($p < 0.05$) lower activity (IC50$=$114$\pm$7 μg/mL) compared to Malik (IC50$=$89$\pm$2 μg/mL) and Snowbird (IC50$=$82$\pm$7 μg/mL). Overall, faba bean had either similar or better antioxidant properties (ABTS, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Iron chelating and Oxygen radical absorbance capacity (ORAC)) than pea and similar or lower antioxidant properties than soy. The antihypertensive properties (Angiotensin-converting enzyme inhibition) of the Fabelle (IC50$=$1348$\pm$148 μg/mL) and Malik (IC50$=$1497$\pm$175 μg/mL) varieties were significantly higher than pea (IC50$=$2222$\pm$376 μg/mL) but lower than soy (IC50$=$639$\pm$21 μg/mL). Similarly, the antidiabetic activity (Dipeptidyl-peptidase-IV inhibition) was higher for soy, comparatively to faba bean and pea. The results suggest that faba bean has an interesting bioactive potential against non-communicable diseases and could be valued as a health promoting food ingredient. Further confirmation of this potential in vivo is required.

**Impact of physical modification on fermentation performance of legume-protein ingredients.** Bibek Byanju, Iowa, State University, USA; Buddhi Lamsal, Iowa State University, USA

The impact of combination of physical treatments (sonication/precooking) on the fermentation performance of some legume-protein ingredients by Lactobacillus plantarum and Pediococcus acidilactici was evaluated. The substrate to water ratio of 1:8 was optimal for the flours of soybean, lentil, and green peas; slurries were first sonicated for 2 and 4 min at 100% amplitude (power density 2.5 W/mL). These physically modified flour slurries were inoculated with Lactobacillus plantarum and Pediococcus acidilactici at 108 CFU/mL and fermented in shake flasks for 72 h at 37°C, with shaking at 200 rpm. The microbial growths and pH were measured at 6, 12, 24, 48, and 72 h. The pH dropped from 6.5 to 4.5 at the initial 24 h and microbial growth was the highest (1013 CFU/mL) at 24 h. The population doubling time for L. plantarum was lowest on green pea (1.3 h) and highest on soybean (1.5 h). The growth rate of L. plantarum improved for the treated substrates but was not significantly different. Similarly, the cell doubling time for P. acidilactici was lowest for precooked lentils (1.1 h) and highest for soybean (1.6 h). Sonication at 2 and 4 min and fermentation of green pea, lentil, and soybean improved the growth rate of P. acidilactici. The protein content and carbohydrate contents of the physically modified and fermented legumes were not significantly different. These physically modified and fermented substrates have the potential benefit as a probiotic protein-rich ingredient thus improving nutritional quality.

**Impact of succinylation on pea protein-curcumin interaction, polyelectrolyte complexation with chitosan, and gastrointestinal release of curcumin in loaded-biopolymer nano-complexes.** Ogadimma Okagu, University of Ottawa, Canada; Jian Jin, Southwest University of Science and Technology, China, USA; Chibuike Udenigwe, University of Ottawa, Canada

This research investigates the effect of pea protein succinylation on the strength and nature of interaction with

curcumin for encapsulation and gastrointestinal delivery. Protein succinylation was accomplished by reacting the pea protein with succinic acid anhydride at 38 oC. pH 10.5. Curcumin encapsulation in both the native and modified protein was achieved by antisolvent precipitation technique and chitosan incorporated by polyelectrolyte complexation method. The biopolymer nanocomplexes obtained were characterized by TEM, dynamic light scattering (DLS), Differential scanning calorimeter (DSC), FTIR, circular dichroism (CD) and curcumin release studied using simulated salivary, gastric and intestinal fluid. Succinylation increased the overall negative charge on the protein from $-34.4 \pm 0.2$ to $-59.9 \pm 0.9$ mV, neutralized the positive charge on the lysine residue and decreased the protein's surface hydrophobicity and binding with curcumin. The binding constant (K) for native protein-curcumin (CUR/PPI) binding and succinylated protein-curcumin (CUR/SPPI) binding calculated from fluorescence quenching were 6.9 ($\pm$ 0.21) $\times$ 104 M$-$1 and 4.2 ($\pm$0.05) $\times$ 104 M$-$1 respectively. In the presence of chitosan, these values increased to 14.5 ($\pm$ 0.64) $\times$ 104 M$-$1 and 187.9 ($\pm$ 12.40) $\times$ 104 M$-$1 for curcumin-native pea protein-chitosan (CUR/PPI/CHI) and curcumin-succinylated protein-chitosan complexes (CUR/SPPI/CHI), respectively. The formation of well-ordered spherical nanocomplexes within the size range of 100–194.5 nm was revealed by TEM. Encapsulation efficiency decreased from 34.65 $\pm$ 0.10% in CUR/PPI to 24.92 $\pm$ 0.03% in CUR/ SPPI and from 85.01 $\pm$ 1.43 in CUR/PPI/CHI to 62.05 $\pm$ 2.95% in CUR/SPPI/CHI nano-complexes. Although polyelectrolyte biopolymers enhance curcumin-loaded complex stability at the gastric phase, the release profile demonstrated that pea protein isolate effectively encapsulated and released curcumin without protein modification or incorporation of chitosan. Therefore, pea protein with or without succinylation or incorporation of chitosan could potentially be used in the encapsulation and oral delivery of curcumin.

**Improving pea protein isolate functionality by phosphorylation with sodium hexametaphosphate.** Yang Lan, North Dakota State University, USA; Jiajia Rao, North Dakota State University, USA

Pea protein isolate (PPI) is commonly extracted using the method of alkaline extraction–isoelectric precipitation, followed by spray drying process; however, PPI prepared from this procedure has limited functionality. In this work, a chemical modification of phosphorylation was added into the procedure and the influence of sodium hexametaphosphate (SHMP)-PPI weight mixing ratios (5:95 and 10:90) and reaction pH conditions (pH 6, 7, 8 and 9) on the structure and functionality of spray-dried PPI was studied. Results showed that PPI has been successfully phosphorylated by SHMP as evidenced by FTIR spectrum and substantial increase in protein surface charge. In addition, the minimum protein content of PPI was 82%, indicating the addition of SHMP did not reduce protein content significantly. Although the secondary structure of $\alpha$-helix and $\beta$-sheet was not significantly impacted by the modification, there are remarkable changes in protein conformation, as evidenced by increased surface hydrophobicity as well as decreased free and total sulfhydryl and disulfide groups. Such structural changes exhibited significant impacts on functionality of spray dried PPI. The solubility of PPI tested at pH 6 and 7 was improved from 75 to 94%, and 90 to 95%, respectively, when SHMP-PPI was mixed at either 5:95 or 10:90, and at reaction pH 9. Additionally, the modification improved foaming capacity of PPI tested at pH 7 from 110 to 120% while there was no significant difference between mixing ratios and reaction pH. Moreover, the modification improved emulsifying activity index of PPI at pH 7 from 43 to 59 m2/g at mixing ratio 10:90. Moreover, the phosphorylation at pH 6 and 9 has significantly improved protein peak denaturation temperature from 85.6 to 92 °C as supported by differential scanning calorimetry, showing improvement in protein thermal stability. These findings provided potentials for facilitating development of pea protein products with better functionalities.

**Inhibition of islet amyloid polypeptide fibril formation by natural polyphenols.** Raliat Abioye, University of Ottawa, Canada; Chibuike Udenigwe, University of Ottawa, Canada

Islet amyloid polypeptide (IAPP), or amylin, is a neuroendocrine hormone that functions in post-prandial glucose metabolism. Aggregation of IAPP results in the formation of cytotoxic fibrils, which plays a role in the loss of $\beta$-cell mass, a common hallmark in type 2 diabetes development. The objective of this study was to investigate the activity of natural polyphenols in inhibiting IAPP fibrillation and observe the role of structural diversity in inhibitor potency. To monitor IAPP fibrillation kinetics in the presence of the polyphenolic inhibitors, Thioflavin T (ThT) fluorescence assay was performed at 1:0.5, 1:1 and 1:2 molar ratios of IAPP-polyphenol. Biomolecular interactions between IAPP and the polyphenolic inhibitors and observation of the changes in IAPP secondary structures upon interaction were evaluated via circular dichroism (CD) spectroscopy. Finally, fluorescence microscopy imaging was used to visually monitor IAPP fibrillation progression in the presence of the polyphenolic inhibitors. Of the 11 polyphenols studied, rutin, caffeic acid, and gallic acid, all of which are structurally diverse from one another, were identified to have inhibitory effects against IAPP fibrillation at a 1:1 concentration. The ThT fluorescence assay suggests phase-specific inhibitory mechanisms of the different polyphenols, indicating a potential role in structure-function relationships. Inhibitory effects of the polyphenols on IAPP aggregation were further confirmed through CD spectroscopy, where a decrease in

β-sheet content of the IAPP-polyphenol samples was observed after 48 hours. Visual confirmation of inhibitory activity on IAPP fibrillation via fluorescence microscopy showed shortened fibrils compared to uninhibited IAPP. Effects of the investigated natural food-derived polyphenolic compounds shows promising results for IAPP fibrillation inhibition, providing new avenues for nutraceutical-based therapies towards mitigating β-cell cytotoxicity.

**Interaction of pea globulin, albumin and glutelin with curcumin and the formation of pepsin resistant and thermo-stabilized well-ordered spherical bionanocomplexes for oral delivery.** Ogadimma Okagu, University of Ottawa, Canada; Jian Jin, Southwest University of Science and Technology, 59 Qinglong Road, Mianyang 621010, China, USA; Chibuike Udenigwe, University of Ottawa, Canada

The impact of structural and physicochemical properties of pea globulin, glutelin and albumin on their interaction with curcumin for their functional role in encapsulation, protection and gastrointestinal delivery of curcumin was investigated. The nature and strength of protein – curcumin interaction was investigated by tryptophan fluorescence quenching technique while curcumin encapsulation with protein was carried out using antisolvent precipitation techniques. The biopolymer nanocomplexes of protein-curcumin were characterized by TEM, dynamic light scattering (DLS), Differential scanning calorimeter (DSC), FTIR, circular dichroism (CD) and curcumin release studied using simulated gastric fluid. The binding constant K for curcumin binding with the alkaline-soluble glutelin (ASF), salt-soluble globulin (SSF) and water-soluble albumin (WSF) fractions was calculated as $2.2\pm0.11\times104$M-1, $2.6\pm0.03\times104$M-1 and $1.3\pm0.15\times104$M-1 respectively. The encapsulation efficiency is consistent with the strength of protein – curcumin binding which consequently depend on the protein surface hydrophobicity. DLS revealed that the encapsulation of curcumin generally increased the size of the protein nanoparticles. DSC showed improved thermostability of the protein on encapsulation of curcumin and TEM revealed the formation of well-ordered spherical nanocomplexes in the size range consistent with that of dynamic light scattering (100–270 nm). FTIR spectroscopy showed prominent spectral shifts of the individual proteins or curcumin in the complex. The various complexes of curcumin-loaded protein fractions showed different curcumin release kinetic under simulated gastric fluid in the presence and absence of pepsin. The curcumin-loaded complexes of ASF (CASF) and WSF (CWSF) demonstrated better curcumin protection in the gastric fluid and hence negligible curcumin release compared to curcumin loaded-salt soluble globulin complex (CSSF). Overall, isolated pea glutelin, albumin and globulin could be a potential candidate for curcumin encapsulation and delivery as demonstrated from the gastric stability of their various complexes, hence ensuring that curcumin reaching the intestinal phase are of physiological relevant amount.

**Modulating intermolecular interactions of pea protein isolate to improve its functional properties.** Yanting Shen, Kansas State University, USA; Yonghui Li, Kansas State University, USA

Proteins exist in numerous spatial arrangements that are stabilized by various intermolecular and intramolecular forces. Different denaturants such as sodium sulfite, urea, sodium dodecyl sulfate, and trypsin can interrupt protein interactions, particularly disulfide bond, hydrogen bond, hydrophobic interaction, and peptide bond, respectively, and thus altering protein secondary and tertiary structures and technological functional properties. The objectives of this study were to investigate the functional properties of pea protein isolate by modulating protein covalent and non-covalent interactions, and understand the physicochemical characteristics (e.g., free amino group, free sulfhydryl, surface hydrophobicity, SDS-PAGE, secondary structure, and SEM morphology) of the modified pea proteins that are responsible for the functional changes. The modified pea proteins were prepared by reacting the protein with sodium sulfite (0.5% and 1%), urea (0.2M and 1M), sodium dodecyl sulfate (0.1% and 0.5%), and trypsin, respectively.

Both urea and sodium dodecyl sulfate modified pea protein had significantly higher water holding capacity and oil holding capacity with up to 5.1 g H2O/g protein, and 3.1 g oil/g protein compared with the control pea protein of 4.1 g H2O/g and 1.3 g oil/g, respectively. Sodium sulfite modified protein also exhibited higher oil holding capacity of up to 2.8 g oil/g. There was no significant difference for the emulsifying properties of the modified proteins. All the denaturation approaches significantly increased protein solubility up to 80 % compared with the control pea protein of 47 % at pH 7. The pea protein hydrolyzed by trypsin possessed the highest solubility (48%) compared with the control (15%) at the isoelectric point (pH 4.5). It also exhibited the highest foaming capacity and better gelation properties than the other modified proteins. This study provides a fundamental knowledge of protein functionalities as related to protein covalent and non-covalent interactions, and will contribute to broader food applications of pea protein.

**Properties of soluble and insoluble navy bean flour components after jet-cooking, soaking, and cooking.** James Kenar, USDA ARS NCAUR, USA; Frederick Felker, USDA ARS NCAUR, USA; Mukti Singh, USDA ARS NCAUR, USA; Jeffrey Byars, USDA ARS NCAUR, USA; Mark Berhow, USDA ARS NCAUR, USA; Michael Bowman, USDA ARS NCAUR, USA; Jill Winkler-Moser, USDA, ARS, NCAUR, USA

Partitioning of pulse flours offers an attractive route to produce novel fractions enriched in valuable components with useful functionality. Navy bean flour was modified by jet-cooking and partitioned by centrifugation into readily obtained water soluble and insoluble fractions. Compositional and physical properties of these fractions were characterized and compared to navy bean flour fractions partitioned by water extraction at 23 °C and 95 °C. Jet-cooking partitioned 49.2% of the flour weight into the soluble fraction that contained 88.8% of the available starch while also solubilizing the highest proportions of phenolics (69.2%) and saponins (58.7%). In contrast, the 23 °C and 95 °C soluble fractions contained only 21.7% and 18.5%, respectively, of the flour weight. The 23 °C-soluble fraction contained no starch and the most soluble protein (45.54 g/100 g) and oligosaccharides (12.4 g/100 g). Swollen starch granules in the 95 °C treatment trapped water soluble components preventing their clean separation into the soluble fraction. Functional properties (color, water activity, water absorption and solubility, viscosity, and foam capacity) reflected starch, protein, sugars, and saponins levels. These results enable development of pulse flour fractions substantially different from raw flour in composition and functional properties.

**Solid-state fermented heat-stabilized defatted rice bran as a source of peptides with anti-melanogenic activity.** Ali Bisly, University of Arkansas- Fayetteville, USA; Navam Hettiarachchy, University of Arkansas-Fayetteville, USA

In cosmetic products, using natural whitening agents to treat skin hyperpigmentation condition is preferable due to the risks of using synthetic inhibitors for tyrosinase, the key enzyme in the melanogenesis pathway and the main cause for the abnormal melanin accumulation. Fermentation, a natural process, mainly Solid-State Fermentation (SSF), has been associated with adding value to agricultural and industrial byproducts as an alternative technology for enhancing the production of bioactive compounds including protein fractions and peptides. Heat-stabilized Defatted Rice Bran (HDRB) is a by-product that is produced after subjecting rice bran to a heat stabilization step to preserve oil quality.

This study was carried out to evaluate the cosmeceutical functionalities of HDRB proteins and peptides (HDRBP) extracted after SSF under optimized conditions: fermentation time of 61h, water content of 41 % v/w, and Bacillus subtilis natto inoculation of 106 CFU/g of HDRB. The ultra-membrane fractionated water-soluble proteins and peptides of SSF fermented HDRB, < 5KDa and 10–5 KDa, showed enhancement in in-vitro tyrosinase inhibition activity when compared with the non-fermented HDRBP fractions: 90.7% and 63.9% for < 5KDa and 10–5 KDa of SSF fermented HDRB, and 12%, and 7% for HDRBP fractions, respectively. The significant increase at ($P \leq 0.05$) in the tyrosinase inhibition activity for the < 5KDa of SSF fermented HDRB fraction with a half-maximal inhibitory concentration (IC50) of 0.41 mg protein/mL was linked to the amount of the small molecular weight component of the peptides.

Based on the results, the SSF can be a cost-effective and efficient approach to enhance the anti-melanogenic bioactivity of HDRB protein and add-value to HDRB in finding applications as a functional ingredient in the cosmeceutical products as a whiting agent.

## Surfactants and Detergents

*Division Vice Chair: Michael Williams, Evonik Corporation, USA*

## Additives 2—Interactions of Surfactants at Solid Surfaces

*Chairs Phillip Vinson, The Procter & Gamble Company, USA; Geoffrey Pasciak, Evonik Corporation*

**Deinking of Printed Plastic Film Using Nonionic Surfactant and Cationic Polymer.** Asmita Baruah, University of Oklahoma, USA; Brian Grady, University of Oklahoma, USA

Plastic production has increased exponentially to 100lbs/year per capita in the world since the 1960's. 18% of the plastic produced are films and 50% of them are pigmented, indicating some color component. Recycling pigmented plastic degrades its mechanical and optical properties which leads to a limitation in its functionality. Alternatively, deinking with surfactants can reduce the residual ink on plastic and increase its tensile properties. Prior work in this field indicated that cationic surfactants were optimal for deinking over a wide range of pH whereas non-ionic surfactants were more cost effective and deinks at basic pH conditions. This research work focuses on deinking plastic film with a combination of non-ionic surfactants and cationic polymer solution with varying pH to decrease costs and find a more environmentally friendly solution. Plastic films were cut into small pieces and the samples were soaked for 24 hours prior to deinking or directly placed in a mechanical agitator at a set temperature after which tensile tests were performed. Deinking with cationic polymer at pka ~7, indicated no deinking at pH 5 after 24 hours presoaking and additional 24 hours agitation time. Slight deinking was observed at similar conditions at pH 12. With a ratio of 1:1 by weight of nonionic surfactant and cationic polymer (high and low pKa polymers), samples were tested at pH 5,7 and 12 to observe deinking and perform mechanical tests to determine changes in tensile properties. The findings of

this research allow for a greater understanding of effective and long-term solution for plastic recycling.

**Incorporation Process of Organic Liquids into Aqueous Geopolymer Suspensions: One-line Torque Monitoring for the Selection of Suitable Alkyltrimethylammonium Bromide Surfactants.** Christel PIERLOT, University of Lille, France; Hanyu Hu, University of Lille, USA; Charles Reeb, CEA, USA; Matthieu Bertin, CEA, USA; David Lambertin, CEA, USA; Catherine Davy, University of Lille, USA; Véronique Nardello-Rataj, University of Lille, USA

Geopolymer has recently emerged as a greener and more sustainable binder for the conditioning of radioactive oils. Mixing organic oils in aqueous geopolymer suspensions causes a torque variation, which gives information on the process. The incorporation of high amounts of organic liquids into geopolymers is apparently possible using suitable surfactants. A series of organic liquids (OL) have been selected for this study, comprising alkanes, paraffinic mineral oils as well as an industrial motor oil and tributylphosphate (TBP), in a view of covering a wide range of viscosity (0.0018 to 2.33 Pa.s). The incorporation of viscous organic liquids ( > 0.2 Pa.s) into fresh geopolymer (GP) results in a regular torque increase, indicating a suitable process with the dispersion of the OL in the form of fine micrometric droplets. When the torque remains stable like TBP/Dodecane mixtures, the incorporation is not possible, or only partially in the form of large droplets quickly coalescing and leading to an organic supernatant above the hardened geopolymer.

In the case of low viscous organic liquids, the effect of using quaternary ammoniums like cetyltrimethylammonium bromide (CTAB) has been studied. Torque monitoring showed that the oil was identically incorporated in the geopolymer slurry whatever the surfactants were introduced at the beginning, through the course or at the end of the process. Finally, torque monitoring highlights that quaternary ammoniums with shorter alkyl chains such as hexamethyltrimethylammonium are able to replace the CTAB with the same efficiency. The powerful action of CTAB on the incorporation of alkanes into GP is therefore due to its positive charge rather than its surfactant effect.

**Isomeric Effect of Linear Secondary Alcohol Ethoxylates on Surfactant Performance.** Sung-Yu Ku, Dow Chemical Company, USA; Wanglin Yu, Dow Chemical Company, USA; Wen Sheng Lee, Dow Chemical Company, USA; Nathan Rau, Dow Chemical Company, USA; Wanda Buckner, Dow Chemical Company, USA; Margaret Whitley, Dow Chemical Company, USA

In alcohol ethoxylate nonionic surfactants, the properties and application of surfactant products are largely dependent on hydrophobe structures. Linear secondary alcohol ethoxylates (SAEs) have demonstrated performance advantages, such as better wetting, less stable foam, and improved handling properties, over primary alcohol ethoxylates (PAEs), and proven to be readily biodegradable.

In an SAE molecule, the ether linkage connecting the polyethylene glycol chain and the alcohol can be varied along the hydrocarbon chain in the alcohol moiety, which causes various isomeric structures. The isomeric structure variations may affect the properties of the resulting SAE surfactant products. In this study, a series of SAE surfactants were produced and the isomeric structures with different ether linkage positions were characterized by NMR techniques. The product samples include those that have the ether linkage positions randomly distributed along the hydrocarbon chain and that have the linkages concentrated at the C-2 position, as well as those with the linkage distribution profiles in between. The SAE surfactant products with different ether linkage distribution profiles were compared for their physical and surface properties, including solubility and gelation behavior in water, surface tension, critical micelle concentration (CMC), dynamic surface tension, wetting properties, and foaming properties. The materials were further compared for their performance on hard surface cleaning.

**More Cleaning and Protection with Less Water via Surface Modification.** Andras Nagy, Evonik Corporation, USA

Novel surfactants and protectants can achieve multiple benefits by changing the properties of various surfaces. These molecules can possess high surface activities, good compatibilities with other ingredients while improving biodegradability for multiple application areas and surfaces. Improvements will be shown in cleaning, protection, aesthetic appearance, biodegradability, water saving and economy in home care, I&I and vehicle care, including ‘‘rinse-free’’ cleaning when limiting use of water becomes essential.

**The Development of Adjuvants for the Removal of Oily Soils and Prevention of Redeposition in Fabric Washing.** Eric Wasserman, Dow Chemical Company, USA; Aslin Izmitli, Dow Chemical Company, USA; Asghar Peera, Dow Chemical Company, USA; Michael Tulchinsky, Dow Chemical Company, USA; Yang Zhang, Dow Chemical Company, USA

Greasy soils, and in particular body oil (sebum), are among the most difficult to remove from clothes during laundering. This problem is exacerbated by the trend toward lower wash temperatures, since some of these soils are partially crystalline at room temperature. We have found that certain alkoxylated amines are well-suited to assist the removal of these soils when added to a typical liquid laundry detergent formulation and tested at near-ambient temperature. There is some

evidence for synergism when mixed with conventional alcohol ethoxylate surfactants. Redeposition of soils already detached from fabric, a common problem also known as ‘‘graying,’’ is also attenuated when these alkoxylates are present.

**Use of Radiotracer Detergency for Mechanistic Understanding of Oily Soil Removal by Nonionic Surfactants.** Kirk Raney, A&I Ventures, LLC, USA

Radiotracer detergency has proven to be a powerful and quantitative method for investigation of fabric detergency mechanisms. In this presentation, a consolidated review is provided of past work with this technique utilizing a variety of oily soils and alcohol ethoxylate surfactants.

Radiotracer detergency from synthetic fabrics was measured using a temperature-controlled Terg-o-tometer and oily soils containing 14C and/or tritium. Single-component nonionic surfactants and pure oils including hydrocarbons and/or triglycerides were utilized in most cases. Phase inversion temperatures (PIT) of the same oil-water-surfactant systems were measured by observing emulsion stability and microemulsion phase behavior at varying temperatures. The kinetics of soil removal during the wash process were also investigated.

Optimum detergency for nonpolar hydrocarbon soils was found to occur above the surfactant cloud point temperature at the phase inversion temperature corresponding to surfactant film composition equivalent to the added detergent composition. For nonpolar-polar soil blends such as hexadecane and oleic acid, much lower optimum detergency temperatures were observed as the polar soil was found to act with the surfactant to form a mixed surface-active film with a low effective HLB. The radiotracer results also showed that the polar component of the soil is removed to a higher extent than the that of the nonpolar portion.

Understanding detergency mechanisms is important for the development of new cleaning systems. In conjunction with phase behavior studies, radiotracer detergency tests have provided a clear relationship between oily soil detergency with nonionic surfactant systems and the relevant PIT. Researchers should consider this technique for future basic studies of detergency and the elucidation of important detergency mechanisms.

## General Surfactants and Detergents: Addressing Surfactant Regulation

*Chair Brian Grady, University of Oklahoma, USA; Julian Barnes, Shell Global Solutions International B.V., Netherlands*

**1,4-dioxane in Alcohol Ethoxylates: Concentration, Measurement Methods and Mechanism of Formation.** Jerrick Juliette, Shell Global Solutions US (Inc), USA; Nathan Fleer, Shell Global Solutions US (Inc), USA; Julian Barnes, Shell Global Solutions International B.V., Netherlands

The New York legislation imposing very low concentration limits on 1,4-dioxane (1,4D) in consumer products after 2022 has stimulated R&D in at least three areas: a) analytical methods to measure 1,4D, b) the surfactant components, and c) the end-use products themselves. This paper describes what Shell is doing in the first two, to help the industry better prepare for the change. This paper reviews the literature on measurement methods for 1,4D, discusses the mechanism for its potential formation and presents data for Shell’s alcohol ethoxylates.

Shell has been involved since the 1970s in the analysis of 1,4D in our alcohol ethoxylates and pioneered the GC-FID method to give a method detection limit (MDL) of 0.5 mg/kg. Since that time analytical methods have improved and the current detection limit for GC/FID, the mainstay of a laboratory supporting chemicals manufacture, is 0.1–0.3 mg/kg. An MDL of <0.1 mg/kg can be achieved with more sensitive R&D-based techniques such as GC-MS with single ion monitoring and isotope dilution. The ACI commissioned the development of such a universal method with a similar MDL for use with finished cleaning products.

With the GC/FID method used at Shell’s manufacturing site in USA the MDL is 0.3 mg/kg and no detectable 1,4D is measured in the alcohol ethoxylate grades. Applying the more sensitive GC-MS method gives an MDL that is substantially lower but is not easily deployed at the manufacturing site.

Mechanistically, 1,4D will not easily form during manufacture of Shell alcohol ethoxylates as a base catalysis method is used and 1,4D requires either a Lewis acid or proton to catalyse its formation. Although mostly excluded, we theorise that trace levels of water may provide the proton to enable back-biting of the ethoxylate chain to form the low level of 1,4D detected.

**Advantages of Branched Alcohol Sulfate (AS) as 1,4 Dioxane Free Alternative for Alcohol Ether Sulfate (AES) and / or Alcohol Ethoxylate (AE) in Cold Water Laundry.** Alan Galuska, Exxonmobil Chemical Company, USA; Jeffrey Harwell, The University of Oklahoma, USA; Brian Grady, University of Oklahoma, USA; Parichat Phaodee, University of Oklahoma, USA; David Sabatini, University of Oklahoma, USA; Joshua Cutler, ExxonMobil Chemical Company, USA; Chunzhao li, ExxonMobil Chemical Company, USA; Shiwen Li, ExxonMobil Chemical Company, Belgium; Sangho Bang, University of Oklahoma, USA

Branches have been known to enhance surfactant performance (increase wetting speed, solubility, hard water tolerance, etc.), but can also reduce essential features such as biodegradability. In this work, we have identified a branch level that maintains a preferred

10-day biodegradability window and low toxicity while boosting many other surfactant properties. C13 alcohols with this optimal branching were then used to make AS, and the performance of this branched AS was compared to commercial linear AS (C12 and C13), AE2S (C12-C14) and AE7 (C12-C15). The branched AS showed substantially improved Kraft T, solubility, wetting speed and hard water tolerance over linear AS. In fact, many of these properties were comparable to linear AE2S and AE7 (without ethoxylation and the emerging regulatory concerns of 1,4 dioxane byproduct). Moreover, this branched AS in 1 to 1 blends with linear alkyl benzene sulfonate (LAS) showed improved low temperature (15°C) detergency for solid non-particulate soils (coconut oil) compared to equivalent AE2S/LAS or AE7/LAS blends at a constant Hydrophilic-Lipophilic Difference of –2.5 (commercially relevant range from prior studies). In fact, the detergency of the branched AS/LAS blend exceeded the detergency of the AES/LAS and AE/LAS blends with much less surfactant. This work suggests that controlled branched hydrophobes may be used to boost surfactant performance while still being readily biodegradable (10-day window), and can be used to produce branched AS, which has advantages over AE2S and AE7 in low temperature detergency without the concern of 1,4 dioxane.

**Amine Oxide Surfactants with Optional Circular Carbon Content Matching Today's Trends and Sustainability Needs in Home Care Formulations.** Elke Theeuwes, Eastman Chemical Technology, Belgium; Stephanie Clendennen, Eastman Applications Innovation, USA; Kristof Moonen, Eastman Chemical Technology, Belgium

Amine oxide (AO) surfactants are widely used in the home care industry because of several advantages they bring to formulators: (i) excellent wetting properties; (ii) superb foam stability quality when combined with anionic surfactants; (iii) exceptional fatty and oily stain removal capability. Together with their broad pH range stability, excellent compatibility with bleaching agents and good environmental profile, AO surfactants are used in a variety of home care solutions.

In this study we investigated the properties of different AO surfactants in home care formulations matching todays trends and sustainability needs.

Besides primary cleaning and hygiene, hard surface cleaners (HSC) can also bring easy cleaning benefits by modification of the surface. Water-soluble acrylic copolymers are added to HSC formulations to make treated surfaces water repelling. We will present data, including contact angle measurements that show how AO surfactants introduce wetting properties to the surface. Compared to surface care polymers AO surfactants have better renewable carbon index and biodegradability.

In laundry solutions, AO surfactants provide exceptional fatty and oily stain removal. This will be showcased by addition of AO surfactant on top of commercial pre-treaters and laundry detergents. Interfacial tension measurements and laundry washing tests provide insight in the cleaning mechanism.

Eastman is supplying the basic raw material, N,N-dimethyl alkylamine, to AO producers and is constantly improving and innovating its tertiary amine platform to give its customers new means of answering changing needs in the AO market. In the past, a switch was made from petrochemical olefin based ADMA to natural fatty alcohol, reducing petrochemical carbon content in AO surfactants below 20%. Now we will introduce technology, based on circular economy principles, that can take out the remaining petrochemical carbon in AO surfactants and help formulators meet their sustainability goals.

**Benefits of NRE Catalyst for Reducing 1, 4 Dioxane Formation in Alcohol Ether Sulfates.** Tamra Weemes, Sasol Chemicals, USA

The state of New York passed a bill that lowers regulatory limits for 1,4-dioxane that can be present in a wide range of consumer products. California is also expected to propose lower regulatory limits soon. The New York bill imposes a limit of 2 ppm 1,4-dioxane by 2022 and 1 ppm 1,4-dioxane by 2023. These new lower limits have a significant effect on the raw material ingredients allowed to be used in the formulated products. Formulators need to understand the contributions each ingredient makes to the total 1,4-dioxane content of the formulation.

1,4-Dioxane is not a significant by-product of base-catalyzed alcohol ethoxylates (AE). However, 1,4-dioxane can be a significant byproduct of the acidic sulfation process to make alcohol ether sulfates (AES). There are several processing and equipment factors that can be used to control the levels of 1,4-dioxane in AES with different degrees of effectiveness and efficiencies. An additional factor that can control the level of 1,4-dioxane created during the sulfation reaction is the catalyst used to produce the ethoxylate for feed to sulfation. Our work demonstrates the effectiveness of using a Narrow Range Ethoxylation (NRE) catalyst during the production of the AE for reducing the amount of 1,4-dioxane formed during the production of the AES. As the SO3 feed mole ratio increases, the NRE-based AE produces less 1,4-dioxane in the resulting AES. When comparing AES on the same mole ratio basis, NRE always produces less 1,4-dioxane compared to KOH-based catalyst. Higher SO3/feed mole ratios produce even more dramatic reductions of 1,4-dioxane.

**Comparison of 1,4-dioxane Quantitation by GC-FID & GC-MS.** Christinna Becktold, Sasol, USA

1,4-dioxane (DO) is a heterocyclic compound that is found in trace amounts in alcohol ethoxylates. Because

it is considered a possible carcinogen, regulations regarding the allowable amount in cleaning and personal care products have become more stringent. Due to the need for consistent reporting of DO levels (below 1 ppm) in our products, a study was conducted to determine the effects of different calibration techniques and detection methods on DO quantitation by gas chromatography (GC). Nine alcohol ethoxylates with varying alcohol chain lengths and degrees of ethoxylation were analyzed by both mass spectrometry (MS) and flame ionization detection (FID). MS analysis was performed using internal standard calibration to mimic our R&D laboratory setup, and FID analysis was performed using external standard calibration to mimic a typical QC laboratory setup. It was concluded that both the MS and FID were able to report reproducible and quantitative data with detection limits below the 1 ppm maximum allowable concentration.

**Influence of Surfactants on Microencapsulation.** Arianne Lopez Hernandez, UPC, Spain

Today, more than 15 % of the people who enter an operating room infected with nosocomial diseases. The main method of transmission is people and gowns. It is intended to give a possible solution through the microcapsules because they have high applications both in the field of medicine, as in the food industry and in detergents.

Once the samples have been made, the aim is to relate different phenomena, like microbiology and physics-chemistry and to be able to impregnate it into a fabric.

The complex coacervation method is used, where first the dissolution of the chitosan and that of the gum Arabic is carried out. The essential oil and surfactant are then added with different concentrations in each sample. For complex coacervation to occur, a change in pH is necessary and the polymer is located around the nucleus that is formed by the essential oil and the surfactant. Finally, the crosslinking agent is added to make the microcapsule more rigid.

It is necessary to impregnate the fabric to give it a longer useful life. The fabric used is cotton and polyester. Once the necessary impregnations have been carried put, the microbiology is carried out. The samples are put in contact with agar and the corresponding bacteria according to the method AATCC.

The samples obtained from a surfactant, Tween 20, and lavender essential oil, formed a miscella and the size was reduced. These conclusions are seen because lavender causes many more interactions, causing more stability. With the impregnation tests, it can be observed that the microcapsules are better impregnated in cotton than in polyester and all the fabrics have worked correctly except polyester. When we use a soluble chitosan, it is diffusion is faster,since it allows the agar water to enter the interior more easily.

## General Surfactants and Detergents: Detergency

*Chairs Julian Barnes, Shell Global Solutions International B.V., Netherlands; Brian Grady, University of Oklahoma, USA*

**Applying Methyl Ester Sulfonate (MES) to Liquid Detergent: Stabilizing MES at Low Temperatures.** Atsunori Morigaki, Lion Corp, Japan; Yasushi kakizawa, LION CORPORATION, Japan

Methyl Ester Sulfonate (MES), derived from sustainable palm oil, has already been put to practical use in powder laundry detergent due to characteristics such as excellent biodegradability and superior detergency even at low surfactant concentrations. However, it remains difficult to apply MES to liquid detergent because of its low solubility at low temperatures. In this study, we investigated new methods of stabilizing MES in liquid detergent at low temperatures as well as the mechanism behind this in terms of micelle structure.

In our experiments, we used C16MES, visually checking the Krafft points of test solutions as they gradually heated from –40oC. We then evaluated the distribution of additives in the MES micelles from the self-diffusion coefficient and determined the position of the additives in the micelles by monitoring, through NMR using DOSY and NOESY, where they were correlated in the MES. Finally, we evaluated micellar size by analyzing SAXS intensities using generalized indirect Fourier transformation method.

The Krafft point of 1% MES solution was about 35oC, which we reduced by adding a 1:1 solution of alkanol amine and aromatic sulfonic acid to the MES. Neither was useful on its own, but the synergetic effects of the different additives efficiently lowered the Krafft point. Monoethanol amine (MEA) and Cumene sulfonic acid (CS) were particularly effective, reducing it to about 20oC. DOSY, NOESY and SAXS suggested that the combination of hydrophilic MEA and CS serves as a hydrophobic complex instead and promotes the distribution of MEA/CS to the micelles, enlarging micelle curvature. This may prevent MES from arranging into a crystalline structure. Furthermore, this complex is effective at lowering the viscosity of MES solution concentrated around 40%, which induces the phase transition from hexagonal to micellar. We deduce therefore that this technique is useful for improving the manufacturing process of liquid detergents.

**Determination of Pectate Lyase Activity in Laundry Detergents.** Franco Pala, Bridgewater State University, USA; Weidong Li, Battelle, USA; Christie Dame, Battelle, USA

Pectate lyase has been utilized in detergent formulations over the past decade to boost detergent

performance in removing stains from fruit and food products containing pectin. Pectin is a class of heterogenous polysaccharides present in the cell walls and intercellular regions of various terrestrial plants. Pectin's chemical structure consists of long-chains of partly esterified alpha(1à4)-D-galacturonic acid units. Other neutral sugars are also present in the pectin structure, mainly as side chains. Extracted pectin is used in the food industry as an emulsifier, stabilizer, and gelling and thickening agent in various products, such as marmalades and dairy products. To properly monitor the evolution of commercial laundry detergent formulations, including the changes in the enzymatic systems complexity and activity, a pectate lyase assay was developed by adapting a pectin identification spectrophotometric method proposed initially by Hansen and coworkers in 2001. Pectin, with a low degree of methylation (Megazyme), was de-esterified before being used as the assay substrate. Pectate lyase present in detergent cleaves the galacturonic chains, forming unsaturated galacturonate fragments, which absorb strongly at 235 nm. A standard solution (0.8 to 7 mU/ml) of pectate lyase (Aspergillus niger, Megazyme) was used for calibration. Pectate lyase enzymatic activity in 1 mg/ml of detergent solution was measured by the initial rate of the formation of unsaturated galacturonate fragments at a temperature of 25 °C and pH 8 (Tris buffer). Pectate lyase activity in detergents was compared to the response of standard solutions. Other enzymes commonly used in detergent formulations, such as protease, amylase, lipase, cellulase, and mannanase, do not interfere with the determination of pectate lyase activity using the proposed method. The pectate lyase activity in selected laundry detergents commercialized in the USA and Western Europe since 2020 measured between zero and 25 U/g-detergent.

**Evaluation of Fragrance and Essential Oil Winsor IV Microemulsions Prepared Using Narrow Range Alcohol Ethoxylates.** George Smith, Sasol, USA; Alicia Huggett, Sasol, USA; Gabe Ortego, Sasol, USA; Christian Jones, Sasol, USA

The incorporation of fragrances and essential oils into home care formulations increases user aesthetics, but they are water insoluble and must be solubilized using surfactants. Alcohol ethoxylates are commonly used as the solubilizing agent due to strong cost performance. Alcohol ethoxylates can be produced using two catalyst systems: KOH, the industry standard, and a narrow range catalyst which is used to peak the oligomer distribution. Peaking the oligomer distribution reduces the amount of unreacted alcohol in the product, lowers the solidification point, and reduces the interfacial tension between oil and water.

Narrow range alcohol ethoxylates are shown herein to be more efficient both at creating Winsor IV microemulsions and at hard surface cleaning than their KOH-derived counterparts. Solubilization efficiency was determined by titrating a solution of 12% fragrance in water using a mixture containing the alcohol ethoxylate. Hard surface cleaning performance was measured using artificial soil applied to white Formica tiles. Soil removal was then determined by optical reflectance as a function of abrasion cycles. Increases in solubilization efficiency and hard surface cleaning performance translate to lower usage and formulation costs.

**FTIR Characterization of Precipitates to Inform Cleaning Product Formulation Decisions.** Claire Dentinger, The Clorox Company, USA; Maria Emilia Perdomo, The Clorox Company, USA; Brian Thompson, The Clorox Company, USA

Fourier transform infrared spectroscopy (FTIR) is an analytical technique that can be used to obtain quick molecular information about samples. When it is combined with attenuated total internal reflection (ATR) sampling this molecular information can be obtained quickly and with minimal sample preparation. In this talk we demonstrate how FTIR-ATR can be used to aid in formulating cleaning products by the quick identification of precipitates. Alkyl polyglucosides (APG) are bio-based surfactants that are often used in formulating cleaning products. In the example discussed here an APG based formula showed the presence of a precipitate after stability testing. Using FTIR-ATR analysis the precipitate was identified giving clear direction to formulation changes that could eliminate the precipitate. Another example of using FTIR-ATR to identify precipitates in cleaning formulation is the quick identification of a siloxane based antifoam that was mistakenly added in excess.

**Influence of Sodium and Ammonium Ions and Their Mixtures on the Properties of Alkylbenzene Sulfonate Surfactant in Aqueous Solutions.** Naycari Forfora, FIRP laboratory, University of Los Andes, Venezuela; Jose G. Alvarado, FIRP laboratory, University of Los Andes, Venezuela; Franklin Salazar-Rodríguez, FIRP Laboratory, University of Los Andes, Venezuela; Ana M Forgiarini, FIRP laboratory, University of Los Andes, Venezuela

Linear alkylbenzene sulfonates (LABS) are the most widely used surfactants worldwide with applications in detergency; nevertheless, their applications have been extended to multiple fields due to their low prices. Their behavior in an oil-water system can be predicted by the Hydrophilic-Lipophilic Deviation (HLD) semi-empirical equation, which considers formulation parameters like salinity, surfactant head and tail, co-surfactant, temperature and oil nature. O/W (Winsor I) and W/O (Winsor II) emulsions are defined by negative and positive HLD values respectively. Particularly, Winsor III systems and microemulsions are represented by an HLD zero value, which presents ultra-low interfacial tension (IFT) and low stability. This condition is usually named optimum formulation for applications in enhanced oil recovery (EOR). In previous studies, it has been shown that

depending on the brine composition different changes can occur, ranging from variations on surfactants aqueous solubility to specific interfacial properties of the surfactant-oil-water (SOW) systems, such as, IFT values. In this study, dodecylbenzene sulfonic acid was neutralized with sodium hydroxide to obtain its respective salt: sodium dodecylbenzene sulfonate (NaDBS). The solubility (at CSURFACTANT = 0.2% w/v) and the critical micelar concentration (CMC) were evaluated with different salts at different concentrations: NaCl, NH4Cl and some of their mixtures. A reduction in the Krafft temperature due to the presence of ammonium ion was found, which is related to an increase in solubility of the surfactant in the brine. In addition, the results show that the ammonium ion in solution reduces both, CMC and optimum salinity (S*) values for NaDBS -heptane-brine systems, in which the minimum interfacial tension is obtained.
In this investigation is determined the synergistic effect of some counterions and their mixtures on surfactants aqueous solubility and interfacial behavior in water-oil systems.

**Viscosity Impacts of Altered Detergent Amine Oxide Structures: variances Observed Due to Dimethyl vs. 2-hydroxyethyl Amine Headgroups and Alkyl Chain.** Eric Theiner, Evonik Industries, USA
Alkyl amine oxides, and lauryl dimethylamine oxide (LDMAO) in particular, have long been used as viscosity modifiers in detergents that also contain anionic surfactants. One benefit that LDMAO will provide is a synergistic thickening effect that is now expected by consumers in premium hand dishwashing detergents. Recently a study was undertaken to examine the differences in this thickening effect when slight changes were made to the base structure of alkyl amine oxides, namely the use of 2-hydroxyethyl groups rather than methyl groups to cap the primary amine as well as the use of an ether amine rather than an alkyl amine. The results were surprising and showed a shifting landscape in a contour plot comparing surfactant and sodium chloride concentration with resulting viscosity. This presentation will discuss the variances in structure and the results in viscosity changes. An attempt will also be made to connect the structural differences with aggregation properties resulting in the shown viscosity changes.

## General Surfactants and Detergents: Surfactant Chemistry, Pure and Applied

*Chairs Julian Barnes, Shell Global Solutions International B.V., Netherlands; Brian Grady, University of Oklahoma, USA*

**Effect of Equilibration Time on the Structural Changes of Bicontinuous Microemulsions near Winsor-iii Liquid-liquid Interfaces.** Douglas Hayes, University of Tennessee, USA; Brian Barth, University of Tennessee, USA; S Venkatesh Pingali, Oak Ridge National Laboratory, USA
Bicontinuous microemulsions (BMEs), self-assembly systems consisting of oil and water nanodomains separated by surfactant monolayers, have many current and potential applications, such as for drug delivery, enhanced oil recovery, separations and purifications and media for the templating of nanomaterials and hosting of multiphasic reactions. In previous work, we discovered through use of small-angle neutron scattering (SANS) that the middle, BME, phase of Winsor-III three-phase microemulsion changes structure in the vertical direction, particularly near the lower, water-BME, liquid-liquid interface. The difference in structure will have significant implications on the performance of BMEs in separations, reactions, and for templating. We observed previously that the extent of the change differed with the equilibration time for the Winsor-III system. We recently completed SANS experiments to better understand the relationship between the structural changes in the vertical direction versus equilibration time (from 30 min to 3 weeks). We have found that, among the parameters associated with the Teubner-Strey model, the quasi-periodic repeat distance (d) was the most sensitive to changes with respect to time and vertical position. The parameter d increased in the downward vertical position and decreased with equilibration times. The increase of d for an isolated BME phase in the downward vertical direction was very strong, at the approach of a BME-glass interface in the sample cell. The increase of d likely represents a higher surfactant concentration at the lower interface of Winsor-III and single-phase BME systems.

**Iron Binding in an Edta-based Gemini Surfactant Monolayer Film.** Matthew F. Paige, Univ Saskatchewan, Canada; David Sowah-Kuma, Univ of Saskatchewan, Canada; Jeveria Rehman, University of Saskatchewan, Canada; Alfred Yeboah, University of Saskatchewan, Canada; Wei Bu, CheMatCARS, The University of Chicago, USA; Ci Yan, Univ of Saskatchewan, Canada
EDTA-based gemini surfactants have been investigated as possible delivery agents for inorganic iron in agricultural applications. While these new compounds hold promise, their performance is imperfect and their mechanism of action in terms of iron-binding remains unclear. To assess the latter, the ability of $Fe3+$ to bind to Langmuir monolayers of a newly-synthesized EDTA-based gemini surfactant has been explored. Sub-phase iron resulted in compacted, liquid-phase monolayers with a mesh-like morphology at the micron length-scale, in comparison with expanded, unstructured liquid-phase monolayers without iron. A combination of surface potential and X-ray reflectivity measurements indicated that $Fe3+$ induced minor conformational

changes in the monolayer, suggesting ionic association to the head group. Direct evidence for the binding of iron was provided by total reflection X-ray fluorescence measurements, which indicated that multiple ions associated with each head group, as opposed to chelating with 1:1 stoichiometry as observed with EDTA in bulk solutions. Cumulative data suggests the adsorption of Fex(OH)y(3x-y)+ complexes with the monolayer surface, as has been reported with other charged and uncharged monolayers. The potential benefit of this binding mechanism on agricultural applications is discussed in context of surfactant design.

**Nano-scale Structure and Composition of Mixed Micelles Revealed by Small-angle Neutron Scattering (SANS) and Molecular Dynamics (MD).** Paschalis Alexandridis, University at Buffalo, The State University of New York (SUNY), USA; Samhitha Kancharla, University at Buffalo, The State University of New York, USA; Dengpan Dong, University of Utah, USA; Marina Tsianou, University at Buffalo, The State University of New York, USA; Dmitry Bedrov, University of Utah, USA
Surfactants encounter various ingredients during their formulation into liquid products and during their use, e.g., upon dilution with water. The interactions of surfactants with compounds such as electrolytes, polar organic solvents and solutes, other surfactants, polymers, and, of course, solvents, especially water, are founded on thermodynamics that reflect intermolecular forces, are expressed in terms of nano-scale organization, e.g., mixed micelles in water, and are manifested in properties such as detergency or thickening. The macroscopic performance of surfactant formulations originates from the nanostructure attained by the surfactants in the presence of solvents and additives. Such nanostructure evolves in response to temporal and spatial changes in the composition and to external stimuli. Thus, it is important to have information available on the nano-scale structure and composition of mixed micelles at various conditions. To this end, we utilize high-resolution complementary experiments (small-angle neutron scattering, SANS, with contrast variation) and modeling (molecular dynamics, MD, employing the Atomistic Polarizable Potentials for Liquids, Electrolytes and Polymers (APPLE&P) force field), and we present here examples on how the structure of micelles formed in water by anionic surfactants (sodium dodecyl sulfate, SDS, perfluorooctanoate, PFOA) responds to the presence of additives (ethanol, urea, polyethylene glycol-based polymers) across a wide range of compositions. A detailed description emerges on how the additives distribute at the outer surface of the micelles and in their interior, which is used to rationalize various properties of the mixtures.

**Solubility of Polyglycol Surfactants in Salt Solutions.** Wanglin Yu, Dow Chemical Company, USA
Managing and enhancing solubility of surfactants in aqueous solutions that contain high concentrations of electrolytes remain a challenge in many applications, including, for example, institutional and industrial (I&I) cleaning and agrochemical formulations. A study on water solubility of a group of polyglycol nonionic surfactants that are varied in both hydrophobe and hydrophile structures in the presence of electrolytes using a cloud point method will be described. Using high throughput methods, a large amount of cloud point data was collected on the surfactants with different structures in various electrolytes, which enabled an empirical model to be developed to predict cloud point change of polyglycol nonionic surfactants in electrolyte solutions. Predicted results by this model were in good agreement with experimental data and enable prediction of the electrolyte tolerance of these nonionic surfactants. The second part of the presentation will focus on the use of hydrotropes to enhance the solubility of polyglycol nonionic surfactants in highly concentrated salt solutions. The effectiveness of the solubility enhancement was highly dependent on chemical structure of hydrotrope. The effectiveness of a hydrotrope to increase the solubility of a surfactant also depended on the EO chain length in the surfactant. The hydrotrope-enhanced surfactant solutions at high concentrations of salt were further investigated by self-diffusion NMR technology to understand the interaction between surfactant and hydrotrope molecules in the solutions. The results suggest that, different from what was observed in a mixed anionic-nonionic surfactant solution, the hydrotropes in this study did not change the packing status of hydrocarbon chains of the nonionic surfactant in aggregates in the solutions.

**Surfactant-loaded Polyelectrolyte/multivalent Ion Coacervates for the Multi-month Release of Antibacterial and Therapeutic Payloads.** Yakov Lapitsky, University of Toledo, USA; Sabrina Alam, University of Toledo, USA; Udaka de Silva, University of Toledo, USA; Jennifer Brown, University of Toledo, USA; Carolina Mather, University of Toledo, USA; Youngwoo Seo, University of Toledo, USA
Complex coacervates (i.e., solute-rich complex fluids formed through associative phase separation) attract widespread interest in controlled release applications. To this end, we have recently shown that poly(allylamine hydrochloride) (PAH) complexation with tripolyphosphate (TPP) forms putty-like coacervates that adhere to diverse surfaces and (by providing high diffusion barriers) can release small molecules over many months. Here we describe how, through surfactant incorporation, these coacervates enable high loadings and multi-month release of amphiphilic and hydrophobic actives; namely, amphiphilic and hydrophobic antibacterial agents and amphiphilic drugs, where the surfactant serves as either the active

ingredient or as a dispersing/solubilizing agent for hydrophobic payloads.

Ionic surfactants, such as the anionic drug, ibuprofen or cationic antiseptic, cetylpyridium chloride (CPC), were concentrated within the coacervates through their ionic association with either the cationic PAH or anionic TPP before the PAH/TPP mixing. When this surfactant association was weak, subsequent PAH/TPP binding displaced the bound surfactant and, even at ~30 wt% surfactant loadings, largely preserved the PAH/TPP coacervate properties. Conversely, when the surfactant binding to the PAH or TPP was strong, it competed with the PAH/TPP association and significantly altered the coacervate properties. The release of both ibuprofen and CPC, however, was sustained over multiple months.

Besides using surfactants as active payloads, surfactant incorporation facilitated the encapsulation and release of hydrophobic actives. Nonionic Tween 20, for instance, was used to disperse the hydrophobic bactericide triclosan in concentrated PAH solutions. Mixing the resulting dispersions with TPP solutions then formed PAH/TPP coacervates that efficiently encapsulated the triclosan. Aside from aiding triclosan dispersion/encapsulation, nonionic surfactant incorporation accelerated its release by evidently enhancing its solubilization. The triclosan release persisted for over 100 days, though (because the release rate ultimately became too slow), the bactericidal effect lasted only a few weeks. Collectively, these findings provide insights on PAH/TPP coacervate use in surfactant-based technologies.

**Use of Tertiary Amine Oxides as Hydrogen Sulfide Scavengers in Industrial Cleaning Formulations.** Greg Cebuliak, West Penetone Inc., Canada

Cleaning formulations for industrial refineries require the use of surfactants, such as amine oxides, to provide surface wetting and foulant removal. Amine oxides have been purported for secondary use as scavengers of hydrogen sulfide gas. The use of tertiary amine oxide as a hydrogen sulfide scavenger in industrial cleaning formulations was investigated and found to provide low-capacity abatement within a neutral pH range of 6 to 8. At normal dilution pH of ≥10, only direct pH-controlled sulfide speciation was found with no mass conversion or direct reduction of hydrogen sulfide. The results were obtained by comparing headspace and aqueous-phase concentrations of sulfide, using GC/SCD and UOP 163 respectively, in treated soured samples against a soured control containing no scavenger treatment. Competing market products were compared to one another in terms of hydrogen sulfide control and found to be markedly different in degree and type of abatement.

## HLD/NAC—Part 1

*Chairs Edgar Acosta, University of Toronto, Canada; Sanja Natali, ExxonMobil Chemical, USA*

**Formulating Detergents with the HLD Expression.** Eric Theiner, Evonik Industries, USA; Stephanie Hochstetler, Evonik Corporation, USA

The Hydrophilic-Lipophilic Deviation expression (HLD) is attractive because of the simplicity of the terms and the manner in which the equation can be used as a model to determine the ideal components to attain the "best" interface, which is to say the lowest interfacial tension between aqueous and oil phases. These systems have been shown to offer, among other things, very effective cleaning performance in detersive systems. Unfortunately, the real world presents issues when one seeks to apply the expression to formulating cleaning compounds or detergents. Commercial surfactants are not pure, aqueous solutions are variable, and soils, which present the oil side of the interface, seem to be infinite in their possible makeup.

This presentation will provide some simplifications to arrive at the needed terms for the HLD expression as well as ideas to show how the "ideal" system is not necessarily needed to achieve good results in cleaning.

**How to Avoid the Current Confusion in Using the SOW Generalized Formulation Expression HLD=0 for Optimum Formulation.** Jean-Louis Salager, FIRP Laboratory, USA; Ronald Marquez, North Carolina State University, USA

In the 1970's enhanced oil recovery intensive studies at University of Texas proposed a numerical equation to relate the interaction of the interfacially adsorbed surfactant and the oil and water molecules. A surfactant chemical potential difference equivalent to Winsor's R ratio was shown to exhibit linearity in many double scan experiments. Around 2000, the currently used basic concept HLD (Hydrophilic Lipophilic Deviation from optimum) was suggested as a generalized formulation tool. The interpretation of the surfactant contribution was proposed to be determined in different ways, sometimes misleading, which have to be cleared. A simple discussion shows how to interpret the old and current experimental data, and how to handle a normalized HLDN to avoid confusions and to use the formulation concept in practice, for many different applications.

**Measuring the Characteristic Curvature of Commercial Ethoxylated Nonionic Surfactants.** Amir Ghayour, Syngenta

Commercial nonionic surfactants are widely used in a variety of industrial and home/personal care applications. Due to distribution in EO groups, they exhibit

changes in hydrophobicity with concentration which becomes a challenge when designing formulations. In this regard, a methodology for conducting phase behavior scans and a model were developed to measure the characteristic curvature (Cc) of commercial nonionic surfactants. The HLD framework and a ln(x) function for Cc vs. concentration were applied to develop the model. Using this methodology, the Cc of nonionic surfactants can be determined at any concentration, quickly and accurately. Subsequently, the developed model was combined with a group contribution model for pure nonionic surfactants to provide insight into the complex behavior of commercial surfactants and the partitioning of oligomers in the excess phases.

**Systematic Approach to Catastrophic Inversions of Epoxy Emulsions.** Matthew Lyon, BASF, USA
Catastrophic inversions are used commonly in industry when formulating stable emulsions of viscous resins (epoxy, alkyd, polymers, etc.). These emulsions are made through changing the water/oil ratio to invert the emulsion and create a stable product. Though well researched in academia, the mechanism is not well understood in industry. A mix of trial and error and reliance on old formulas is standard which can limit innovation and keep costs high. Presented will be a look at how using HLD provides a clear path to obtain the fastest inversion while also minimizing the emulsion size.

## HLD/NAC—Part 2

*Chairs Sanja Natali, ExxonMobil Chemical, USA; Edgar Acosta, University of Toronto, Canada*

**Effect of Alkyl Ethoxylates Polydispersity on the Hydrophobicity with Surfactant Concentration.** Sanja Natali, ExxonMobil Chemical, USA; Edgar Acosta, University of Toronto, Canada
The surfactant hydrophobicity for commercial alkyl ethoxylates have been found to depend on the degree of polydispersity in the number of polyethylene oxide groups in the chain. This work introduces a recent bifunctional model for polar oils to explain the experimental trends. Overall, at low surfactant concentrations, a greater fraction of the ethoxymers with lower number of ethylene oxides (Low EON) tends to partition into the bulk oil phase, leaving the high EON surfactants at the interface. These effects are reported with branched alkyl ethoxylates and its impact on the formulation of various products is discussed.

**HLD Modeling of the Concentration-dependent Behavior of Nonionic Surfactant Mixtures.** Gregory Dado, Stepan Company, USA; Rachel Lang, Stepan Company, USA
Surfactant-Oil-Water (SOW) systems that comprise commercial alcohol ethoxylates can exhibit optimal formulation (the Hydrophilic-Lipophilic Deviation (HLD) $= 0$ condition) that is substantially dependent on surfactant concentration. An approach towards addressing this dependency within HLD modeling is to replace the Characteristic Curvature term, which has been generally treated as a constant for a given surfactant, with an "Effective Characteristic Curvature" function. In this paper, the application of this modeling approach towards nonionic surfactant mixtures will be reported. SOW phase-inversion temperature (PIT) data have been modeled using linear mixing rules that treat component surfactant characteristic curvature terms as functions of concentration rather than as constants. In at least some cases, linear mixing rules enable accurate predictions of SOW PIT values for systems comprising combinations of two commercial ethoxylate surfactants, for example combinations of branched alcohol ethoxylate with nonylphenol ethoxylate. This modeling approach has been applied to successful prediction of non-linear PIT behavior vs. mole fraction test surfactant in the so-called PIT-Slope experiment, which is an approach to measuring surfactant hydrophobicity on the basis of test surfactant addition to a reference SOW comprising a fixed amount of C10E4 reference surfactant. These results have important implications to the viability of applying reference SOW perturbation to the interpretation of PIT-Slope results, as well as to the suitability of SOW perturbation in generating characteristic curvature parameters for commercial nonionic surfactants.

**Predict Fragrance Oil Droplet Size in Personal Care Liquid Product Using the HLD-NAC Principle.** Hongwei Shen, Colgate-Palmolive Company, USA; Changlong Chen, Colgate Palmolive, USA; Bor-Jier Shiau, The University of Oklahoma, USA; Jeffrey Harwell, The University of Oklahoma, USA; Suzanne Jogun, Colgate Palmolive, USA; Ewelina Lesniak, Colgate Palmolive, USA; Douglas Mooney, Colgate Palmolive, USA; Cheryl Kozubal, Colgate Palmolive, USA
Fragrance compounds play an important role in consumer products, both for aesthetic reasons and for their antimicrobial properties. Yet, incorporation of fragrance compounds into liquid products sometimes could trigger sudden change of appearance of the product, such as inducing undesirable haziness.
In this work, solubilization behavior of fragrance in surfactant liquid system is successfully predicted based on the hydrophilic-lipophilic deviation (HLD) principle and net-average curvature (NAC) model. First of all, the equivalent alkane carbon number (EACN) of 4 targeted fragrances, and the corresponding k, Cc parameters of a ternary surfactant system were determined experimentally from microemulsion phase behavior using a reference extended surfactant, alkoxy sulfate. The HLD

parameters of the ternary surfactant mixture, consisting of an anionic, a zwitterionic, and a nonionic surfactant, were also calculated separately based on the ideal mixing rule; the result is consistent with that determined experimentally. For further verification, a series of Winsor Type I (Oil-in-Water) microemulsions of fragrance oil species in surfactant systems were then created. The resulting oil droplet size was calculated based on the NAC model. The calculated size values matched well with the droplet size measured from dynamic light scattering. Impact of co-solvent ingredients dipropylene glycol and isopropyl myristate on fragrance behavior was also investigated.
The HLD-NAC model shows robustness in predicting fragrance oil solubilization in complex surfactant systems. The HLD-NAC model has the potential to reduce tedious lab tests, save materials, and improve product development efficiency.

**Recent Advances in the HLD-NAC Framework.** Edgar Acosta, University of Toronto, Canada; Rafael Perez-Franco, University of Toronto, Canada; Brandon Cordeiro, University of Toronto, Canada; Carol Tan, University of Toronto, Americo; Boza-Troncoso University of Toronto, Canada
This presentation reviews recent advances in the HLD-NAC framework, starting with a revisit of the concept of characteristic curvature (Cc) and the use of the curvature interpretation of the HLD to determine the surfactant hydrophobicity parameter ''sigma''. The second aspect reviews the effect of surfactant concentration on the sigma or Cc parameter for alkyl ethoxylates, interpreted in light of the recent bifunctional polar oil model. The third and last aspect of this review revisits the concept of equivalent alkane carbon number (EACN), in the view of the phase behavior of light alkane oils.

**Utilization of Binary and Ternary Inorganic Salt Mixtures with HLD-NAC.** Michael Warren, The University of Oklahoma, USA; Bor-Jier Shiau, The University of Oklahoma, USA; Jeffrey Harwell, The University of Oklahoma, USA
Understanding how to address mixtures of inorganic salts is important to enhancing or optimizing formulations used in consumer products, personal care products, cosmetics, enhanced oil recovery and environmental remediation. The objective of this work was to employ microemulsion phase behavior of reference surfactants of both anionic and nonionic character using inorganic salts; specifically, the anions chloride (NaCl, KCl, CaCl2) and sulfate (Na2SO4, K2SO4, MgSO4). Finding the optimum salinities, Smix*, of defined binary and ternary mixtures provided shifts in the amphiphilic behavior that was modelled using the Hydrophilic Lipophilic Deviation (HLD)/Net Average Curvature (NAC) framework. HLD parameters K, Cc, and temperature coefficients ($\alpha$t, ct) will be presented along with comparing the predictions of salt models found in literature. This work has further provided evidence that the cation is of primary interest for the studied surfactants whereas changes in the anion showed minimum difference.

## Next Generation Ingredients

*Chairs Scott Backer, Home and Personal Care, USA; Nancy Falk, The Clorox Company, USA*

**Biological Answer to True Odor Removal.** Donna Nguyen, Novozymes, USA; Sarah Shepherd, Novozymes, USA; Jatin Sharma, Novozymes, USA; Renata de Souza Hyczy, Novozymes, USA
Reblooming of odor has been one of the most challenging aspects in laundry cleaning. Until now, formulators had to rely on a combination of perfumes and odor scavengers to mask odors while the underlying problems, body soils, remained trapped deep within the fabric fibers. Recent advancements in enzymatic technology have shown to remove the build-up of body soils and provide a biological solution to true odor removal. Furthermore, in a recent study conducted by Novozymes, consumer panelists showed a clear preference for this novel technology when washing their apparel and home textiles.

**Dow Components for Antimicrobial Formulations.** Afia Karikari, Dow, USA; Daniel Mayfield, Dow, USA; Christine Schultz, Dow, USA; Kelly Ann Embiscuso, Dow, USA
Dow ingredients help home care, industrial and institutional product manufacturers create more effective and sustainable cleaning solutions. Our expansive product portfolio for antimicrobial formulations include solvents, surfactants, chelants and polymers can play an important role in the removal of soils from surfaces, which is often the first step in creating effective antimicrobial formulations.

- Surfactants to lower the surface tension and assist in the wetting and spreading of the active.
- Solvents to aid in the dispersion and dissolution of soils which helps apply the disinfectant to the surface.
- Chelants to help to control divalent cations to help remove tough stains like soap scum and hard water and keep surfactants more soluble, improving performance.
- Polymers that act as dispersants and inhibit scale and soil redeposition, which enables cleaning without residue.

In this presentation, we will showcase how we have combined our advanced formulation expertise with the market knowledge and high throughput capabilities to design products that can be used as inerts to meet the urgent need for disinfectant formulations.

**High Performing Concentrated Surfactant Solutions.** Art Sutton, BASF Corporation, USA; Michael Capracotta, BASF Corp, USA; Ashish Taneja, BASF Corporation, USA; Scott Walters, BASF Corporation, USA; Stephen Gross, BASF Corporation, USA; Simone Bright, BASF Corporation, USA; Kevin Salmon, BASF Corporation, USA

The global pandemic has dramatically impacted the detergent market. Unprecedented demand for detergents has stretched supply chains and changing consumer needs have led to new consumer habits including more reliance on e-commerce to purchase cleaners. BASF has developed new detergent solutions to meet new consumer needs and help detergent manufacturers streamline their raw material management. In this presentation BASF will highlight how new Dehypound® Surfactant blends can serve as chassis for a variety of cleaners, including e-commerce friendly concentrates. Application data will also highlight both the strong cleaning performance and versatility of these new solutions.

**Innovative Bio-based Surfactants for Cleaning Product Applications. When Sustainability Comes with Cost Effective Performance.** Tony Bartolini, Arkema, France

By working closely with our partners to understand and to answer current consumer needs combined to ARKEMA's DNA based on Innovation and Sustainability, a new range of non-ionic bio-based surfactants is now coming to the market. Key factors such as product provenance, labelling, performance and competitiveness were considered during the development having in mind the Ideal surfactant range for cleaning applications.

Sustainable provenance of the innovative range comes with a new vegetable source out of traditional impacting food chain routes and without impact on deforestation.

Readily biodegradable, with low toxicity and with non-corrosive labelling, it allows to develop concentrated cleaning formulations with limited label impact.

The innovative range brings cleaning performance to another level by offering a combination of a unique degreasing power and low foaming benefits matching requirements of cleaning products as hard surface cleaning and automatic dishwashing.

This easy to use and highly concentrated surfactant range provides multiple benefits and can enhance cleaning formulations to meet today's formulator and consumer needs while limiting the impact on the environment.

**MAGNATHOX Ethoxylates for Industrial Applications.** Ramesh Varadaraj, Sasol North America, USA; Derek Rensing, Sasol, USA; Dustin Landry, Sasol, USA; Jennifer Bickel, Sasol, USA; Ollie Normand, Sasol, USA

Sasol has recently introduced a new line of ethoxylates under the trade name MAGNATHOX. This presentation will illustrate some of the unique properties of MAGNATHOX ethoxylates and detail some of the industrial applications that the ethoxylates are being used for now and others that have proven promising in lab trials. The MAGNATHOX line consists of eight ethoxylates based on Sasol's C20–30 alcohol and vary in degree of ethoxylation from 3 to 150 moles of ethylene oxide. The heavy alcohol base imparts unique properties to MAGNATHOX ethoxylates which make them promising candidates for use in multiple application spaces. While already in commercial use in agricultural applications, this ethoxylate line has proven effective in IPC, home and personal care, and Oil and Gas applications. A highlight of select applications that take advantage of MAGNATHOX ethoxylates and their unique chemistry will be explored.

**New Cellulontm Cellulose Liquid: Improved Cationic-compatibility for Providing Reliable Suspension in Fabric Softeners and Personal Care Products.** John Swazey, CP Kelco, USA; Janet Akande, CPKelco, USA

In personal care and fabric care applications, there is a need to find compatible and biodegradable structuring agent options that can provide stability for the suspension of particulates in cationic systems such as fabric softeners and hair conditioners. Most fabric softeners and rinse-off personal care systems contain high enough cationic levels that make them incompatible with many nature-based agent offerings. The goal to successfully formulate these cationic systems with sustainable structurants for suspending components has proven challenging. This is due to limited options of structuring agents that are nature-based, biodegradable, and able to deliver suspension-performance with long-term storage stability.

CP Kelco has developed a cationic-compatible version of CELLULONTM cellulose liquid, a Fermentation Derived Cellulose (FDC) product. CELLULONTM cellulose liquid is produced by the fermentation of syrup-based media with the bacterium, Komagataeibacter xylinus. CELLULONTM cellulose liquid FDC fibers are produced as a 3-dimensional, reticulated net-like structure with very high surface-area by weight ratio, enabling it to create significant yield point for reliable suspension at relatively low use levels. CP Kelco's cationic-compatible CELLULONTM cellulose liquid is readily biodegradable, nature based and easy to use. Cationic-compatible CELLULONTM cellulose liquid is very efficient in liquid fabric softeners and can provide stable yield values adequate to provide long-term stability for the suspension of particulates such as opacifiers, pearlescents, and fragrance encapsulates. Stability for transparent systems is even possible. Additionally, cationic-compatible CELLULONTM cellulose

liquid is able to maintain the stability of particles for suspension without significantly affecting viscosity, and future variants of CP Kelco's cationic-compatible CELLULONTM cellulose liquid could contribute to pour viscosity if desired.
As a nature-based and biodegradable product, CP Kelco's cationic-compatible CELLULONTM cellulose liquid FDC is available in a ready-to-use liquid form. No pH adjustments or high shear mixing is required for its use.

## Performance Additives

*Chairs David Stott, Mary Kay, Inc., USA; Robert Nolles, Cosun Biobased Experts, USA*

**Compatibility of Hydroxyproline Rich, Natural Proteins (HRPs) and Surfactants in Hard Surface Cleaner Formulations.** Eric Yezdimer, Gelita USA, USA; Nina Rittereiser, Gelita AG, Germany; Ceren Yüce, Gelita AG, Germany; Berthold Köhler, Gelita AG, Germany; Matthias Reihmann, Gelita AG, Germany
Quartz crystal microbalance (QCM) analysis and contact angle measurements have found that dilute solutions of hydroxyproline rich, natural proteins (HRP)s form net hydrophilic layers, a few nanometers thick, on different surfaces. These layers are protective and support surfactants, thus preventing soil materials from directly contacting the surface and allowing for easier sequential cleanings. In practical cleaner applications, the chemistry and concentration of the surfactant utilized dictates their potential interspersion within the protein layers.
Surface contact angle experiments of a series of sulfate, alcohol ethoxylate and glucamide surfactants with similar alkyl chains were performed. The results indicate that surfactants with stronger and/or smaller polar head groups appear to have a stronger affinity for HRPs than surfactants with larger, more diffuse polar head groups. Increasing concentrations of HRPs in solutions of surfactants comprised of anionic or smaller non-ionic polar head groups show an initial increase in contact angle followed by a decrease. Surfactants containing more diffuse head groups show minor or slight increases in contact angle with increasing protein concentration.
The effect of the surfactant sodium dodecyl sulfate, known to denature proteins through binding of its alkyl chain with protein hydrophobic regions and its sulfate group with positively charged amino acids, on the thickness of HRP layers was also explored with QCM. The results indicate layers of protein and surfactants form on the surface. Rinsing with water or dilute surfactant remove parts of the layers, although a thin, higher affinity protein layer remains.
The effectiveness of cleaning formulations with ionic and non-ionic surfactants, with and without HRPs, were evaluated using a scrub abrasion and washability tester. These experiments found that formulations with HRPs provided for easier sequential cleaning with all surfactants studied. The results demonstrate that HRP protective layers appear compatible with several common hard-surface cleaning surfactants.

**IFF's Perspective on Testing Methodology Improvements to Enhance Detergent Differentiation.** David Hong, International Flavors & Fragrances, USA; Arjen Hoekstra, DuPont Nutrition & Biosciences, Netherlands; Marvanne DeClerck, DuPont Nutrition & Biosciences, USA
Consistent performance improvements have been made over time to detergents. Enzymes contribute significantly to improved performance and enable a more sustainable laundry process by their ability to remove a broad range of consumer-relevant stains under consumer-relevant conditions, including more sustainable, lower temperature washing. Detergent performance is often assessed using regional industry test protocols. However, industry test protocols do not always properly assess the benefits of enzymes in detergents or lack discriminative power that is necessary to optimize detergent performance.
Recently, there have been efforts in Europe to update and improve the stain set used in the detergent test protocol. In North America, the ASTM method currently specifies a broad stain set and allows for flexibility in choosing stain manufacturers or handmaking stains. It is important to consider the stain composition and manufacturing method as these will influence the stain's degree of reproducibility, sensitivity, and discriminative power. Using the combination of consumer market research and historical experience with technical and consumer-relevant monitors, DuPont works with suppliers and customers to develop differentiating stain sets for assessing enzymatic performance in detergents. The selected set of stain monitors will help detergent brands to demonstrate how their products can deliver robust performance and differentiated cleaning to meet their consumer needs.

**New Low-VOC Solvent for Aqueous Cleaning Formulations.** Scott Jaynes, Croda Inc, USA
Formulators of safe, sustainable cleaning products have limited choices when choosing a water compatible solvent. Traditional glycol ethers can have measurable VOC's or toxicity concerns and are petroleum-based. D-limonene is plant-based, but also has VOC concerns and is difficult to solubilize. More recently developed green solvents meet some sustainability criteria but often provide only moderate cleaning performance or may have other drawbacks such as bad odors or aquatic toxicity. We now introduce a new class of ester-

based solvent with high compatibility in aqueous cleaning formulations, excellent grease cutting performance, and a safe and sustainable product profile. The products are readily biodegradable, non-irritating, VOC-exempt and exhibit low aquatic toxicity. Performance testing of the solvents at only 0.5–2% in aqueous cleaning formulations exhibited significant improvements in the removal of greasy soils versus surfactant-based cleaners alone. Benchmarking against competitive technologies showed the cleaning performance to match or exceed other available solvents on a variety of greasy or mixed soils. The new solvents were found to be effective in applications including household and institutional cleaning, industrial degreasing, and oil and gas cleaning operations. This new green solvent provides an opportunity to replace high VOC, high solvent cleaners with bio-based, sustainable aqueous formulations that still deliver excellent cleaning performance.

**Preventing Odor When Drying Clothes Indoors.** Masanori Fushitani, Lion Corporation, Japan

In recent years, Japanese consumers have become increasingly conscious of hygiene. They want to prevent odors in household laundry, especially when drying indoors. This is also a potential issue in Asia and Europe where there are opportunities for indoor drying. Such unpleasant odors come from the metabolizing of soiling such as sebum and protein, which provide nutrition to bacteria. Therefore, in order to eliminate these sources of odors, we have developed technologies to remove sebum and protein from clothes as well as the technology to suppress bacteria growth during indoor drying. Moreover, we have identified Moraxella and Brevundimonas as odor-producing bacteria during indoor drying and applied antibacterial technology to laundry detergents. However, we have not achieved complete consumer satisfaction regarding odor. Therefore, in order to reconfirm the factors involved in odor dissatisfaction, we examined this dissatisfaction in relation to soiling on towels. The result was that we detected a certain amount of polysaccharide on unsatisfactory towels in addition to the sebum and protein. Furthermore, the amount of polysaccharide was correlated to the strength of the odor. Accordingly, this study reports on the causes of this polysaccharide as well as technology to remove it.

**Probiotic Cleaning—Shifting the Paradigm.** Michael Furlan, Novozymes, USA; John Harp, Novozymes, USA; Kevin Luo, Novozymes, USA

Humans spend more than 90% of their lives indoors. In our homes and offices, we are exposed to dirt, debris and microscopic life that become trapped in these indoor spaces. We are often overly enthusiastic about making these environments as clean as possible and ultimately this involves killing the microbes around us with harsh chemicals. However, people are becoming more aware of scientific evidence showing the importance of microbes in human health. Consequently, consumer demand for natural cleaning solutions which support a more balanced microbial home environment continues to increase. Novozymes aims to shift the way we think about what it means to clean by harnessing the natural cleaning power of probiotic microbes.

In this study, we tested the efficacy of a probiotic cleaning technology that achieves a long-lasting clean and supports the diversity of microbes in our homes. The key to developing this type of next generation product is to have a cleaner where chemistry and microbes work in harmony. Replacing harsh chemistry with natural ingredients that promote probiotic survival will result in products that can remove soils from household surfaces immediately and continue to clean microscopically where traditional cleaners may not be able to act. We will share data that demonstrates how chemistry can impact probiotic spores in a formulation, how active probiotics excrete enzymes for cleaning, and how microbial technology responds to the presence of soils on a surface. By better understanding these aspects of probiotic cleaners, we can develop technology to shift the paradigm of cleaning

**Single Unit Dose Laundry Detergents: Development and Evaluation of New Formulations.** Daniel Piorkowski, Henkel Corp, USA; Casey Camire, Henkel Corp, USA

Evaluation strategy and techniques for development of new Single Unit Dose Laundry Detergent to ensure proper stability and performance of new materials (solvents, surfactants, polymers). Identification and elaboration of common failure points.

Methods of evaluation include film to liquid compatibility testing, water activity, pac stability testing and pac dissolution; among others.

Results will be combined to show a conceptual formula that is optimized for a specific application that enables the highest possible stability at certain cost/processing constraints, while maximizing performance.

Presentation will conceptually show the thought process and techniques for new formulation development for Single Unit Dose Laundry detergents and how certain chemistries may not be compatible with current systems and may require additional optimization.

## Personal Care 1: Skin Care

*Chairs Hongwei Shen, Colgate-Palmolive Company, USA; Anthony O'Lenick, Nascent Technologies Corporation*,

**Development of an in Vitro Test for the Skin Irritation Potential of Mild Surfactants and Its Validation Against Human Patch Testing.** Fiona Jacobs XCellR8, United Kingdom; Carol Treasure, XCellR8, United Kingdom

‘‘Existing in vitro tests for skin irritation were historically developed for analysis of harsh chemicals and do not have the sensitivity required to resolve differences between today’s ultra-mild surfactants.
Traditional and novel mild surfactants (0.3%, pH4.7) were applied to 3D skin models. Extension of the dosing time from 18 to 48 hours allowed subtle differences between mild and ultra-mild formulations to be quantified. The surfactants were ranked in order of irritation potential using the ET50 value (time taken to reduce skin model viability to 50%). We demonstrated predictive capacity of the test by direct validation against human patch test data to provide a Clinical Score (CS).
Initial validation results ranked, in order of irritation potential: SLS > SLES > CAPB > novel mild surfactant. Blends of SLES and CAPB were subsequently tested. Rank order of ET50 values correlated with CS and, importantly, matched the results of the same blends in commercial formulations.
We have now generated a bench-marking database allowing results to be interpreted in the context of typical industry ranges for specific surfactant-containing product types across the personal care spectrum. The test provides an effective pre-screen for formulations and ingredients relevant to the personal care industry. When using this test in the initial stages of product development, it can identify the mildest ingredient combinations, therefore reducing the number of patch test volunteers required and their risk of developing an adverse irritation reaction.
Here we describe the optimisation of an in vitro method using reconstructed human skin models, to detect small differences in mildness to human skin and importantly, the validation of the method directly against human patch tests, building confidence that the in vitro results are predictive of clinical score and providing a reference database for the interpretation of results when testing raw materials and formulations.

**Influence of Surfactant Type on the Stability of W/si Suspoemulsions for Cosmetic Applications.** Lizeth Paola Lopez Belcorp, Colombia; Ana Forgiarini, Universidad de los Andes de Venezuela., Venezuela; Johnbrynner García, Belcorp, Colombia; Edwin Baquero, Universidad Nacional de Colombia, Colombia
Formulators of cosmetic and personal care products face great challenges when developing new products with various sensory and efficacy attributes, with the primary objective of ensuring system stability over the life of a product. Surfactant as a stabilizing agent is primarily responsible for the stability and performance of many daily products. For this reason, a study on make-up foundations was carried out evaluating the stability of a pigmented wáter-in-silicon suspoemulsion, W/Si, with mineral photoprotection. Stability evaluation was performed for samples with varying concentrations of three separate emulsifiers with similar HLB values, but different chemical nature. Make-up foundations were manufactured in concentrations of 1, 3, 5 and 7 wt% for each of the emulsifiers: A) PEG-10 dimethicone, B) PEG/PPG-18/18 dimethicone in dimethicon and C) a mixture of polyglyceryl isostearate-4, cetyl PEG/PPG-10/1 dimethicone and hexyl laurate. In all cases, stability was estimated using photo-centrifuge dispersion analysis, rheological oscillatory shear tests, and X-Ray separation analysis. The results obtained showed that although HLB value indicates similar hydrophilic/lipophilic balance, the structure and nature of surfactant is the determining factor in regards to stability of suspoemulsions. Long-tailed siloxanes emulsifiers (A and B) showed an improved effect on stability of the studied system when an increment of concentration was carried out, while an increment of aliphatic-tailed emulsifier (C) concentration in the formula decreased stability of the system. Furthermore, the polymeric nature of emulsifiers A and B has an effect on the viscosity of the system, especially when the amount of monomeric units in the polymer increases which leads to increasing viscosity. This viscous effect is crucial for systems where avoiding sedimentation of suspended solids is necessary as is the case for make-up foundations.

**Oils of Our Largest Organ, the Skin. their Chemistry and Contribution to Skin Health.** Apostolos Pappas, Nogra Pharma and Entrinsic Biosciences, Switzerland
‘‘The skin is the largest organ of the human body. Skin oil synthesis is fundamental for skin functions. Their chemistry and biology seem unusual, as many skin lipids are not found in other tissues within the human body. These surface lipids are of sebaceous and keratinocyte/epidermal origin.
Triglycerides, waxes and squalene are secreted by the sebaceous glands and are deposited via the hair canal on the surface of the skin. Sebaceous oil is involved in the pathogenesis of acne, seborrheic dermatitis, oily hair as well as hair loss, since unique, complex and unusual desaturated fatty acids, waxes and unusual squalene accumulation are unique manifestations for hair biology and health. In addition, more evidence has been advocating that essential dietary fatty acids, their metabolites are influencing the hair follicle and fur in several preclinical models. Understanding the roles of skin lipids is fundamental for decoding the basic physiology of healthy skin and hair.
Epidermal lipids as ceramides are fundamental for the skin barrier’s properties. Recent studies have demonstrated the involvement of the epidermal oil barrier in eczema and dry skin conditions. A general practice for cosmetic chemists is to create formulations that they will proximate skin’s natural barrier components, integrity and physiology. Hence, understanding the physical and biochemical elements of skin’s barrier are pivotal for designing the best and functional skincare products.

Ceramide biomimicry could give insights and further dissect the best strategies that one needs to account and create the best efficacy through targeted formulations

Information on the oils of the largest organ of our human body are essential to anyone in the industry who wishes to come with better formulations and products from skincare to detergents and from hair care to surfactants that help to retain skin beneficial oils and remove the ones that contribute negatively on Skin Health

**Plant Lipid Classes in Skin Care.** Benjamin Schwartz, AAK USA, USA

This will be a brief overview of the different classes of plant-based lipids used in skin care products as a means of replenishing the barrier function of the epidermis.

We will discuss triacylglycerols/triglycerides (fats and oils), wax esters, terpenes, sterols/sterol esters, fatty acids, phospholipids, and ceramides. Additionally, as we go through these categories, we will briefly touch on some of the differences between the composition of native skin lipids and the corresponding plant-based versions used in skincare products.

**Significance of Surfactant Selection in Skincare Sensory. Mark Chandler, ACT Solutions Corp, USA**

Objective/Hypothesis: Formulators of skincare emulsions have 3 objectives: Stability, Sensation, and Superiority. The first is a given and takes skill to accommodate the challenges of an ever-changing consumer marketplace and fears over the safety of many (safe) ingredients. The third is usually handled by cosmetic active ingredients which ultimately assist in making the skin look more attractive. The sensory part has been seen as the easiest of the three, approached primarily by mixing and matching emollient oils for intended tactile sensations. This presentation will challenge this notion and challenge formulators to get better at the basics of making oil and water peacefully coexist, and to be bold in exploring alternative ways to make this happen.

Methods Used: Expert panels from Sensory Spectrum were used to evaluate roughly 30 sensory attributes of emulsions. Both emollient oil and emulsifier were varied in the study outlined by Wiechers. Discussion on emulsion stabilization mechanisms and formulation techniques are taken from a lifetime of study of the work from such notables as Tadros, Friberg, Griffin, Dederen, Klein, and others.

Results: Emollient selection has influence on the After-feel of skincare emulsions, but surfactant emulsifier selection has control of Appearance, Pick-up, and Rub-out, plus an equal influence to emollient on After-feel.

Conclusions: Formulators of skincare emulsions need to get better at understanding the range of emulsifiers and stabilization mechanisms available to them.

**Synergy Between Hydrotropes and Surfactants for the Aqueous Solubilization of Fragances.** Véronique RATAJ, Centrale Lille Institut, France

Fragrances are present in numerous day-life products, either as actives in fine perfumery or as additives in personal and home care products. In fine perfumery, they are generally solubilized in aqueous ethanol while in formulated products, their solubilisation is ensured by surfactants and hydrotropes. One challenge for perfumers today is to replace ethanol by water. One way to promote the solubilization of hydrophobic fragrances in water is micellar solubilization using surfactants. However, they are not entirely satisfactory due to residues they can leave on the skin and clothes and to the tacky feel they give. On the other hand, hydrotropes avoid these drawbacks but the high amounts required to solubilize significant amounts of fragrances (»10%) hinder their use for such an application. However, by taking advantage of remarkable synergies occuring between surfactants and hydrotropes, these amounts can be considerably reduced making the systems more attractive for potential applications. To address this issue, we have developped a series of bio-based solvo-surfactants which are short chain (C3-C5) nonionic ethers derived from glycerol and isosorbide with well-defined properties to meet the specifications. Their self-association and synergy with surfactants and fragrances has been investigated by DLS/SLS. The resulting fragrance/water/ hydrotope/surfactant microemulsions are stable over a large range of temperature, transparent, fluid and contain less than 2% of surfactants.

References

[1] V. Nardello-Rataj, J-M. Aubry, Microémulsions aqueuses volatiles de parfums et d'huiles essentielles basées sur l'utilisation de solvo-surfactifs. Brevet Fr 12/03187, Université Lille1, 2012.

[2] T. Lukowicz, R. Company Maldonado, V. Molinier, J-M. Aubry, V. Nardello-Rataj, Fragrance solubilization in temperature insensitive aqueous microemulsions based on synergistic mixtures of nonionic and anionic surfactants. Coll Surf A, 2014, 458, 85–95

## Personal Care 2: Hair Care and Cleansing

*Chairs Anthony O'Lenick, Nascent Technologies Corporation, USA; Hongwei Shen, Colgate-Palmolive Company, USA*

**Critical Formulation Requirements for Cleansing and Active Deposition from Shampoos.** Manuel Gamez-Garcia Ashland Specialty Ingredients, USA

An analysis of the processes of cleansing and active deposition from shampoos shows that for these two phenomena to occur simultaneously the active must be part of a unique phase composed of surfactants,

auxiliary deposition ingredients, and a polycation. The composition and features of this unique phase will vary depending on type of active; for instance, the unique phases of silicone, fragrance, medicated ingredients, or of any other oil soluble ingredient display between them completely different characteristics. Yet, a common important aspect to all these unique phases is their process of formation which always occurs by a flocculation process involving the active, auxiliary ingredients, and a coacervate. The latter, which acts as the flocculating agent, results from the interactions between polycation and anionic surfactants during the shampooing process. The main feature in all these unique phases is, however, their ability to undergo effective hydrodynamic filtering by overcoming critical boundary layer phenomena at the substrate surface. Thus, for cleansing and deposition to occur simultaneously a delicate balance between all participants is necessary. A discussion of important polycation and surfactant properties will be presented.

**How to Achieve Greater Sustainability in Hair Care Conditioning with Enhanced Performance: Rescued by Ester Quats.** Brian Yang, Evonik Corporation, USA; Anne Mu Evonik Corporation, USA; Jochen Kleinen, Evonik Operations GmbH, Germany; Joachim Venzmer, Evonik Operations GmbH, Germany

Ester-quats are commonly used as softening agents in textile/fabric care. In contrast, their use in personal care as conditioning agents is not as popular. One reason is that commonly used alkyl-quats have better formulation stability and cost/performance properties. However, the growing sustainability trend is tilting the landscape towards greater use of ester quats for their biodegradability and ecotoxicity profiles, which overall are better than alkyl-quats. However, ester-quats are prone to hydrolysis at common formulation pH. Another task is achievement of a high active content while keeping the product in a favorable liquid form from a processing point of view. By carefully choosing the hydrocarbon chain moiety, we were able to produce a product in liquid form with 100 percent active content and better pH stability. The product showed comparable, if not better, conditioning properties than the benchmark, Behentrimonium Chloride. Formulation-wise, surprisingly, the material is not only suitable for cream rinse conditioner as one would expect, but also shows some benefits in conditioning shampoo.

**Investigation of Sulfosuccinate with Varying Hydrophobe Structures.** Christian Jones, Sasol, United States; George Smith, Sasol, United States; Alicia Huggett, Sasol, United States; Gabe Ortego, Sasol, United States

This research evaluates the role of the hydrophobe on anionic sulfosuccinate surfactants. Sulfosuccinates were made on lab scale size and compared to one commercial sample of dioctyl sulfosuccinate surfactant (DOSS). Surface activity and appearance of a surfactant molecule can be altered by the hydrocarbon tail. Hydrophobes investigated include hexanol to dodecanol linear and branched structures. General surface chemistries were evaluated from critical micelle concentrations to surface tensions, while limited application testing was also performed.

**Quantifying Technical Changes in the Properties of Hair After Treatment with Products and Ingredients.** Trefor Evans, Tri-Princeton, USA

The remarkable structure of our hair is degraded by our everyday existence. Most notably, the wear and tear associated with grooming and styling provides constant abrasion and fatiguing; while more dramatic 1-off cosmetic treatments (e.g., coloring, perming, relaxing, etc.) are considerably more harmful. The well-being of our hair throughout this process is the foundation of a multibillion dollar, marketing-lead industry. But, at the same time, the functionality of these products is firmly rooted in the field of colloid and surfactant science. This presentation will describe well-established experimental approaches to assess how well ingredients and products are able to beneficially improve hair qualities. Such efforts help drive the product development process by providing tangible, quantitative comparisons; while simultaneously offering the potential for attractive consumer communication messages (i.e., 10x stronger; 5x softer). Yet a major complicating factor involves the difference between ‘‘consumer language’’ and ‘‘scientific language’’. As will be illustrated, there is danger in taking consumers’ words literally.

**Structure-property Relationships and Application Efficacy of a Series of Alcohol Sulfates.** Gabe Ortego, Sasol, USA; George Smith, Sasol, USA; Alicia Huggett, Sasol, USA; Christian Jones, Sasol, USA

As part of ongoing investigation into the use alcohol sulfates, a series of products were synthesized via the falling-film sulfation of both linear and branched fatty alcohols through a range of molecular weights. The resulting molecules were analyzed for both physical properties and performance in established use scenarios in order to discover trends in structure-property relations and to identify high-performing species for industrial use. Products were analyzed for structure confirmation, and their surface properties analyzed (critical micelle concentration, surface tension, interfacial tension). For application testing, the series of sulfates were tested for efficacy as dioxane-free foam boosters, foam stabilizers, wetting agents, and detergents. The results of this work identify a selection of alcohol sulfates as potential substitutes for commonly used dioxane-containing surfactants, and demonstrate the effects hydrophobe branching has on an alkyl sulfate’s performance.

## Regulatory Issues

*Chairs Yvon Durant, Itaconix Corporation, USA; Kathleen Stanton, American Cleaning Institute, USA*

**Change Is in the Air: A Shifting Regulatory Paradigm for Fragrances.** Darci Ferrer, Fragrance Creators Association, USA

Regulations of fragrances throughout the U.S. supply chain are changing and drastically. Since the passage of the Frank R. Lautenberg Chemical Safety for the 21st Century Act in 2016, new regulations of fragrances are being considered and implemented at unprecedented rates. Rather than settling the U.S. chemical framework for fragrances, there's been an explosion of state activity. But as the states consider fragrances, both within and beyond the bounds of TSCA with ingredient disclosure, new challenges have arisen given the compositional complexity of fragrances. Some new rules threaten reformulation without available alternatives. That, along with pressure for greener alternatives, is driving the fragrance industry to innovate. However, once new, greener molecules are developed, manufactures are finding it difficult to obtain regulatory approval to commercially produce their fragrances. These regulatory pressures are occurring during a backdrop of a pandemic, when fragrances are an essential part of the cleaning product and disinfection supply chain. This presentation will provide attendees with a clear understanding of the current and future state of the regulatory paradigm for the U.S. fragrance industry and opportunities for the industry.

**Hazard/risk Assessment Approach for "New" Chemical Submissions Under TSCA.** Edwin Bisinger, Itaconix Corporation, USA

A key part of the U.S. Toxic Substances Control Act (TSCA) is the requirement that "new" chemical substance must be evaluated by the U.S. EPA prior to commercialization in the United States. In 2016, TSCA was amended or "reformed" by the Lautenberg Chemical Safety Act and the U.S. EPA is now required to make an affirmative finding on the safety of each "new" chemical before it is allowed into the marketplace. Therefore, each company intending to commercialize a "new" chemical substance in the U.S. should have a hazard/risk assessment process in place that transparently documents the safety of the "new" chemical substance. Adequately documenting safety can potentially reduce regulatory restrictions when commercializing "new" chemicals under TSCA. This process should also incorporate a green chemistry discussion.

**Impact of Brexit on Chemical Compliance in Europe.** James Lloyd, H2 Compliance, United Kingdom; Beth Bidstrup, H2 Compliance, USA

With the end of the Brexit transition period and the signing of a Free Trade Agreement between the UK and the EU, what does this mean for chemical compliance in Europe? What about the impact of the Northern Ireland Protocol? Completely separate regulations means a duplication of requirements to enter the market of Great Britain, albeit with "transitional provisions" to ease the way forward in the short-medium term. We'll walk through the new requirements to enter the UK market, and similarities and differences to the EU market requirements, focusing predominantly on REACH, CLP, and the UK equivalents.

**Impact of Continued TSCA Implementation on Surfactants and Detergents.** Thomas Berger, Keller and Heckman LLP, USA

The Toxic Substances Control Act (TSCA) (15 U.S.C. § 2601 et seq.), initially enacted in 1976, is the general chemical control law in the United States. TSCA regulates the manufacture, import, processing, distribution, use, and export of non-exempt "chemical substances," including detergents and surfactants. TSCA is administered by the U.S. Environmental Protection Agency (EPA).

The "Frank R. Lautenberg Chemical Safety for the 21st Century Act" (LCSA) was enacted on June 22, 2016, and significantly modified TSCA. The LCSA provided for modified and new authority for EPA to review and regulate new and existing chemicals in U.S. commerce. TSCA provides for the testing (section 4), premanufacture notification (section 5), regulation (sections 6, 7), and reporting (sections 8, 12, and 13) of new and existing non-exempt chemicals. Although the LCSA amended the majority of the substantive sections of TSCA, the law's changes to section 5, 6, and 8(b) have had the most significant practical impact. EPA's post-LCSA section 5 premanufacture notification (PMN) process continues to evolve, the Agency continues to prioritize, assess, and regulate "high priority" chemicals under section 6, the TSCA section 8(b) Inventory "reset" requirements impose new obligations on "inactive" chemicals, and under section 26 EPA has begun assessing fees and is in the midst of amending TSCA fee requirements.

Violations of TSCA are subject to enforcement and substantial monetary penalties. In addition, TSCA noncompliance can require immediate cessation of commercial activities. TSCA compliance is further complicated by complex and often unpublished nomenclature rules.

**Non-animal Testing: What We Can and Can't (Yet) Do in Vitro to Ensure Product Safety, Consumer Appeal and Regulatory Compliance.** Carol Treasure, XCellR8, United Kingdom

Progress in non-animal testing has seen significant acceleration over the past decade. Consumer pressure has led to a number of bans and restrictions on animal testing that have fueled this explosion of activity, leading to technical advances and regulatory approvals that may previously have seemed impossible.

As an industry, we are on a continual journey to evolve more human-relevant science to ensure safe products, and to translate these technologies into practical, regulatory-compliant solutions. We are still a long way from our desired destination.

This talk will provide a concise overview of which non-animal safety tests have regulatory acceptance, as well as non-regulatory methods that can be used in weight-of-evidence approaches, providing valuable information for exposure-led risk assessment of consumer products.

Importantly, we will highlight where some key challenges remain. We will discuss how collaborative approaches are needed to overcome them, ensuring robust science to create safer-than-ever products, satisfy the regulators and meet the increasing ethical expectations of today's global consumers.

**The Regulatory Landscape of Consumer Products Containing 1,4-dioxane.** Kathleen Stanton, American Cleaning Institute, USA

Legislators and regulators have taken greater notice of chemicals and contaminants in products as consumer right-to-know (RtK) laws continuing to proliferate. One chemical of note to surfactant suppliers and users is the by-product 1,4-dioxane as several categories of consumer products contain alcohol ethoxysulfates (AES) and alcohol ethoxylates. Not only are formulators facing disclosure requirements, but some jurisdictions are also considering 1,4-dioxane concentration thresholds for products. This presentation will detail the activities at federal and state levels, and how industry is responding.

## Surfactant Mixtures and Trace Components

*Chairs Sukhwan Soontravanich, Ecolab, USA; Ronald Marquez, North Carolina State University, USA*

**Effect of Trace Components in Surfactant Systems on Interfacial Properties.** Dominique Langevin, Laboratoire de Physique des Solides, Université Paris Saclay, France

It is well known that minute amounts of surface-active species significantly affects the behavior of many surfactant solutions. The origin of this effect will be discussed with the help of measurements made with various experimental methods: equilibrium surface tension, dynamic surface tension, neutron reflectivity, surface rheology including surface viscosity, micelle lifetime. The changes in interfacial properties lead to important changes in the properties of surfactant systems, such as phase diagrams for instance. We will discuss the example of foams properties and show that minute amounts of hydrophobic solutes can considerably slow down foam drainage and foam coarsening. Recent foam coarsening experiments performed in microgravity conditions in the International Space Station will be presented. The slowing down of drainage and coarsening is due to changes in different surface parameters, respectively surface viscosity and surface layer permeability.

**Ethylene Oxide Distribution in Industrial Alcohol Ethoxylates.** Nimesh Poddar, Sasol, USA; George Smith, Sasol, USA; Tommy Hamilton, Sasol, USA

Fatty alcohols—linear, branched, and/or their mixtures—of even- and odd-carbon numbers are reacted with ethylene oxide (EO) to produce alcohol ethoxylates (AE), widely used as nonionic surfactants in domestic and industrial applications. The hydrophobe structure, i.e., linear vs branched, carbon-chain length; the degree of ethoxylation, the hydrophilic component; and the EO distribution greatly impact the properties of AE, the applications they are employed in and their biodegradability. To better understand the properties and performance characteristics of AE—synthesized from a variety of alcohols, carbon-chain lengths of > C12; comprised of varying moles of EO; and using different catalysts—an analytical study is undertaken to determine the EO distribution within these products. Because the detector response for individual ethoxymers can vary greatly and because reference standards of single-cut ethoxymers of > 9 moles are not readily available, derivatization procedures based on high-performance liquid chromatography (HPLC)—coupled to ultraviolet-visible detection and mass spectrometry—have been devised previously to overcome these challenges. Based on the AE structures, one or more of these HPLC methods are employed to determine the EO distribution within them and will be reported here.

**Evaluation of a Single Quad GC/MS Technique for Trace Analysis of Pahs in Beauty Products.** Jeanne Hankett, BASF Corp, USA; Jennifer Holtz, BASF Corp, USA

European restrictions on the presence of polycyclic aromatic hydrocarbons (PAH)s in consumer products have vastly increased due to demonstrated negative human health effects. The European Food Safety Authority defined a list of priority PAHs of concern in 2008. The European Chemicals Agency published a 2019 guideline on the restriction of PAHs in consumer products. A new need has emerged to quantify regional lists of restricted PAHs at low levels in complex matrices.

Small changes in the chemical makeup of PAHs can greatly affect their toxic properties but PAH mixtures often span hundreds of different compounds. The similar molecular structures of PAHs are difficult to distinguish using traditional techniques like single-quadrupole gas chromatography-mass spectrometry (GC/MS). As such, triple-quadrupole detectors (QQQ) have become the gold standard for trace-analysis of PAHs at ppb

levels. However, GC/MS-QQQ instrumentation is expensive and requires additional method development. In cases where time or available equipment is a factor, it is advantageous to utilize the cheaper single-quad instrumentation.

We present an investigation of a single-quad Agilent PAH Analyzer system to quantify region-specific PAHs in industrial beauty products: 13 PAH compounds in two surfactants, and 16 PAHs in vitamin E-acetate. Our approach translated a method for measuring PAH compounds regulated by the USEPA to less volatile EU priority PAHs. We successfully quantitated all 13 PAH compounds in surfactants with sub-ppb reporting limits. One PAH could not be resolved from matrix interference. We also quantified 12 of 16 PAH compounds of interest in vitamin E-acetate with sub-ppb reporting limits. Results demonstrate that when applying a strategic method, quantitation of individual PAHs on a single-quad GC/MS system is possible at sub-ppb levels and that interferences with PAH values are highly matrix-dependent. For certain matrices, application of single quad GC/MS may be used to quantify trace PAHs without a sample cleanup step.

**Importance of Dialkyldimethyl Ammonium Chloride / Polyoxyethylene Alkyl Etherinteractions: Application to Detergent/disinfectant Formulations.** Véronique RATAJ, Centrale Lille Institut, France; Loïc Leclercq, Université de Lille, France; Gaétan Rauwel, Laboratoires Anios - an Ecolab Company, USA

Surfactant mixtures are used in numerous applications since they generally exhibit superior performances resulting from better interfacial properties than individual surfactants. Actually, in most cases, binary surfactant systems exhibit synergism both in surface tension reduction and in mixed micelle formation. Such synergetic effects are important for a wide range of surfactant-based phenomena. Cationic surfactants like didecyldimethylammonium chloride [DiC10][Cl] are well-known biocidal agents while nonionic polyoxyethylene alkyl ethers CiEj, are useful as detergents and wetting agents. However, the mixing of both these surfactants can lead to important modifications of their biocidal and detergent activities which may be altered. Within this context, mixed aggregate formation and synergistic interactions of binary surfactant mixtures of [DiC10][Cl] with CiEj ($i = 10, 12$, $j = 4, 6, 8$) have been investigated for various [DiC10][Cl]/CiEj ratios by several methods including tensiometry, pulsed field gradient NMR spectroscopy and a [DiC10]-selective electrode. Diffusion coefficient measurements of micelles confirmed the synergistic interaction between the surfactants. It is shown that the formation of surface monolayers and mixed aggregates from [DiC10][Cl]/C10Ej mixtures are driven both by tail/tail and head/head interactions whereas [DiC10][Cl]/C12Ej co-aggregation is mainly driven by tail/tail interactions. Hence, the co-aggregation phenomenon notably influences the biocidal activity of [DiC10][Cl] on the Candida Albicans fungi. In the presence of C12Ej, the biocidal activity of the cationic surfactant is inhibited due to its trapping in the mixed aggregates whereas in the presence of C10Ej, the biocidal activity of the surfactant mixture is maintained. The mode of action is also confirmed by a faster increase of the zeta-potential of a Candida Albicans suspension in the presence of [DiC10][Cl]/C10E8 than in the presence of [DiC10][Cl]/C12E8. Therefore a judicious adjustment of the alkyl (i) and polyoxyethylene (j) chain lengths of CiEj avoids the observed antagonistic effect on the biocidal activity of [DiC10][Cl].

**Quantification of Free Alcohol Content in Industrial Alcohol Ethoxylates by 1h NMR.** Chan Park, Sasol, USA

Ethoxylation of alcohols results in valuable chemicals widely used in various applications such as Agrochemical, Oil & Gas, Inks Paints & Coatings, and Home Care. To control the quality of the product, monitoring and quantifying free alcohol content in alcohol ethoxylates is crucial. Traditionally, free alcohol in ethoxylates is quantified by gas chromatography (GC). However, more complex or high temperature GC methods are required for the ethoxylates of mixture of alcohols (three or more) or heavy alcohol (chain length > C20). Herein, the quantification of free alcohol content in such alcohol ethoxylates via simple proton nuclear magnetic resonance (1H NMR) method is presented. In this method, the alcohol ethoxylate is derivatized with trichloroacetyl isocyanate (TAI), which selectively replaces the -OH group with more electron withdrawing isocyanate group. As a result, the peaks for ethoxylates and free alcohol are shifted and become distinguishable. This is not typically achieved via the traditional GC method alone. Further analyses of the derivatized sample by homonuclear correlation spectroscopy (COSY) NMR and GC along with control experiments confirm that 1H NMR is a quick and reliable method to quantify free alcohol in complex alcohol ethoxylates.

**Using the Dynamic Transitional Inversion as a Tool to Track the Optimal Formulation. Application to EOR.** Jesus Ontiveros, Centrale Lille Institute-ENSCL, France; Guillaume Lemahieu, FORMULACTION, USA; Valerie Molinier, Total, USA; Jean M. Aubry, ENSCL, USA

In chemical Enhanced Oil Recovery (EOR), surfactant mixtures must be optimized to obtain the so called "optimum formulation" characterized by a three-phase behaviour (WIII) and an ultra-low interfacial tension. To attain this condition, discontinuous formulation scans of Surfactant-Oil-Water (SOW) systems are usually performed until equilibrium. In this work, the conditions in which the dynamic transitional inversion of Surfactant/Oil/Water emulsions and equilibrium data of Surfactant/Oil/Water microemulsions matches are described,

using both temperature or salinity as formulation variables.
The dynamic phase inversion temperature (PIT), initially described for systems based on ethoxylated non-ionic surfactants, proves to be relevant for systems based on typical EOR non-ionic/ionic surfactants mixtures and it allows determining the optimum formulation of crude oil based systems[1]. Dynamic salinity phase inversion (SPI) was used with well-defined surfactants and oils and, as the PIT, the inversion of emulsified systems is very close to the ''optimum salinity'' when the Water Oil Ratio is in the vicinity of 1. The SPI is used to track the ''optimal formulation'' using a mixture of an ionic EOR surfactant (sulfated propoxylated n-alcohol) and a nonionic co-surfactant (ethoxylated i-alcohol), with or without alkali and crude oil[2]. This continuous salinity scan of the stirred SOW system significantly accelerates the optimization of surfactant blends suitable for a given oil reservoir.
[1] G. Lemahieu, J.F. Ontiveros, V. Molinier, J.-M. Aubry, Using the dynamic Phase Inversion Temperature (PIT) as a fast and effective method to track optimum formulation for Enhanced Oil Recovery, J. Colloid Interface Sci. 557 (2019) 746–756. http://sci-hub.tw/10.1016/j.jcis.2019.09.050.
[2] G. Lemahieu, J.F. Ontiveros, N. Terra Telles Souza, V. Molinier, J.-M. Aubry, Fast and accurate selection of surfactants for enhanced oil recovery by dynamic Salinity-Phase-Inversion (SPI), Fuel. 289 (2021) 119928. http://sci-hub.tw/10.1016/j.fuel.2020.119928.

## Surfactants and Detergents Poster Session

*Poster Chairs Julian Barnes Shell Global Solutions International B.V., Netherlands*

**Applying Methyl Ester Sulfonate (MES) to Liquid Detergent:Improving the Physical Properties of MES for the Manufacturing Process.** Kazuki Shimizu, Lion Corporation, Japan; Yoshihiro Kimura, Lion Corporation, USA; Hiroshi Kimura, Lion Corporation, USA; Fumiya Niikura, Lion Corporation, USA; Taku Nishio, Lion Corporation, USA
Addressing environmental problems is one of corporate management's most important tasks to achieve a sustainable society. Since the 1980s, we have been developing MES, anionic surfactant made from sustainable palm oil and have used it in powder detergent. MES is plant-derived surfactant, it has been confirmed by life cycle assessment that it emits less carbon dioxide compared with petro-derived anionic surfactant, such as Linear Alkyl benzene Sulfonate, and Alkyl Ether Sulphate. 1)
In 2012, responding to changes in the global clothing detergent market, we began selling liquid detergent formulated with C16/18MES in Malaysia. Formulating liquid detergent with C16/18MES means heating and dissolving C16/18MES flakes, so efficiently manufacturing liquid C16/18MES detergent in a range of locations, including cold places, requires improving C16/18MES solubility by developing a highly soluble, fluid C16/18MES solution.
First, we focused on the Critical Packing Parameter (CPP) and attempted to improve the physical properties of C16/18MES by adding small-CPP surfactants in order to increase the curvature of the C16/18MES molecular arrangement and expand its lamellar phase. We observed a correlation between the additives' CPP values and improvement in physical properties, especially with the addition of C12/14MES and C12/14 Methyl Ester Ethoxylate.
Second, we selected C12/14 MES, which is the same surfactant type as C16/18MES, as an additive and optimized the ratio of C16/18MES and C12/14MES based on concentration, fluidity point (temperature) and detergency. By doing this, we succeeded in developing a C12/14/16/18MES solution with excellent concentration and fluidity while maintaining the same detergency as C16/18MES. Furthermore, we found that by using an alcohol as a second additive, we could maintain transparency and fluidity even when increasing the MES concentration to 40wt%.
1) Hiroyuki Hagiwara et.al, ''Reduction of CO2 emissions using palm-based MES as laundry detergent'', Malaysian Palm Oil Board International Palm Oil Congress (2009).

**Characterization of glycerol monooleate and water based liquid crystalline structure phase transitions induced by variation in temperature and water content.** Shweta Mistry, Ryerson University, Canada
The amphiphilic properties of polar lipids in the presence of water are the driving force for mesomorphism and self-assembly during the formation of liquid crystal (LC) structures. The complex structures of LCs can be useful in various food science, pharmaceutical and biotechnical applications, such as drug and bioactive compound delivery. Herein, I have studied the formation of different LC structures such as lamellar, cubic, and hexagonal phases at different ratios of glycerol monooleate (GMO) to water and characterize their temperature-dependent phase transitions using polarized light microscopy, small angle X-ray scattering (SAXS), rheology, and Raman spectroscopy. My research sheds light on the fundamentals of this phase transition behaviour; specifically how the rearrangement of the biocompatible lipid GMO molecules, due to changes in molecular interactions caused by temperature induced transitions and water content, relates to their functional properties, such as the viscoelasticity of the LC gel. I have been able to correlate the change in the Raman spectra of the samples at different temperatures with the change in the SAXS Bragg spacing ratios and the change in storage and loss modulus of the gel while

gradually heating the samples at 0.2°C/min. I am able to observe and characterize LC transitions from lamellar to cubic to hexagonal phase.

Correlation between hydrophilic-lipophilic deviation (HLD) and spontaneous imbibition for oil extraction from rapeseeds. Yancie Gagnon, Université de Technologie de Compiègne (UTC), France; Houcine Mhemdi, UTC-ESCOM, USA; Frédéric Delbecq, UTC-ESCOM, France; Elisabeth Van Hecke, UTC, France

The new Surfactant-Aqueous Extraction Process (SAEP) for oil extraction from oilseeds has been directly linked to the hydrophilic-lipophilic deviation (HLD) parameter since its inception. The well-known HLD method has shown its effectiveness in finding the lowest interfacial tension formulation in order to reach the highest oil solubilization in an aqueous phase. However, in SAEP, the role of the solid material (oilseeds) should be considered as well. Obviously, the efficiency of SAEP also depends on the ability of the surfactant-solution to spread over the seed surface, but also to penetrate into it and to remove the oil from it. All that deals with wettability and imbibition ability. In this line, imbibition methods have been proposed in the literature related to crude oil to investigate the liquid (oil and aqueous phase) displacements from the solid, either spontaneously or by using centrifugal forces. At first, the solid-surface is saturated by the oil, then the surfactant-solution is mixed with the oil-saturated solid. Spontaneous and forced displacements are recorded over time. Three correlation models have been proposed in the literature: Amott, Amott-Harvey, and USBM.

This work is devoted to investigate the spontaneous imbibition process of rapeseeds by different surfactant-aqueous solutions formulated according to the HLD method. For this purpose, the HLD formulation method was coupled with the Amott method to better understand the impact of the formulation (optimal surfactant and salt concentrations) and the operating conditions (extraction time, centrifugal forces) on the oil removal. Results led to a better understanding of the behavior of the complex water-surfactant-oil-seeds systems. Several useful correlations were identified and discussed in relation to SAEP performances.

**Dispersion of hydrophobed silica particles by adsorption of asphaltenes extracted from a Venezuelan crude oil.** Franklin Salazar-Rodríguez, FIRP Laboratory, University of Los Andes, Venezuela; Ana Díaz, University of Los Andes, Venezuela; Jose Delgado-Linares, Colorado School of Mines, USA; Johnny Bullón, University of Los Andes, Venezuela

Asphaltene adsorption into solid and liquid substrates has been linked to several processes of crude oil production such as enhanced oil recovery (EOR), heavy oil emulsification for transportation purposes, foam stabilization and dehydration. The amphiphilic character of the asphaltene molecules favors their interfacial interactions and modifies the energy of the involved interfaces. Asphaltene adsorption into solids is of critical importance for practical applications since the wettability of the solid surface might be changed and thus its properties. Phenomena like dispersion of solid particles in aqueous and oil phases may be significantly affected by the presence of adsorbed asphaltenes. In this work, the hydrophobation of silica particles (D50=28.59 mm) by adsorption of asphaltenes separated from a Venezuelan heavy oil was studied. Properties such as wettability (Washburn method) and sedimentation rate (spectrophotometric method) were evaluated. From the adsorption isotherm, two critical hydrophobation thresholds (0.093 mg/m2 and 0.368 mg/m2) were found out; these two thresholds are lower than the surface saturation concentration. The low rate of capillary rise of water is an evidence of the sudden wettability change of silica particles induced by the adsorption of asphaltenes. Likewise, asphaltenes generated a reduction in the sedimentation rate of hydrophobed silica particles in kerosene due to the increase in the particle-solvent interactions.

**Oil drop deposition and rheology of methyl ester sulphonate based surfactant systems in relation to hair shampoo application.** Emily Tan, KL-Kepong Oleomas Sdn Bhd, KLK OLEO, Malaysia; Rumyana Stanimirova, Sofia University, Bulgaria; Veronika Yavrukova, Sofia University, Bulgaria; Gergana Radulova, Sofia University, Bulgaria; Elka Basheva, Sofia University, Bulgaria; Krassimir Danov, Sofia University, Bulgaria; Peter Kralchevsky, Sofia University, Bulgaria; Hui Xu, TPOZ, KLK OLEO, China (People's Republic); Yee Wei Ung, KLK OLEO, Malaysia; Jordan Petkov, KLK OLEO, United Kingdom

Conditioning shampoos serve a dual purpose: cleaning and conditioning hair. Their main ingredients include surfactants (for removal of sweat and dead skin cells), conditioning agents (which are deposited as oil drops on hair to ensure washed hair is soft, tangle-free, and frizzy-free), and cationic polymers (to mediate between the surfactants and conditioning agents).

Surfactants used in hair shampoo are usually anionic. They adsorb and bring negative surface charges to the oil drop and hair. The resulting electrostatic repulsion suppresses oil drop deposition. To overcome this undesired effect, cationic polymers are added. Surfactants and cationic polymers form joint aggregates in bulk solutions and are present in the wetting films intervening between the oil drop and hair. Their interactions play an important role in determining the amount of oil drop deposition. The absence of either result in minimal oil drop deposition. High oil drop deposition is desirable as it means that less conditioning agents will be washed away during rinsing.

In this study, oil drop deposition was investigated using different surfactant systems: sodium lauryl ether

sulphate (SLES) or methyl ester sulphonate (MES) with cocamidopropyl betaine (CAPB). The results showed that increasing the fraction of CAPB in the surfactant mixture suppressed oil drop deposition, MES provided easier drop adhesion than SLES, and addition of NaCl enhances, whereas cocamide monoethanolamine (CMEA) suppressed oil drop deposition.

Further studies were conducted to investigate the rheology of mixed solutions of MES and CAPB. Rheological profiling provides useful information that can assist in predicting consumer sensory evaluation and flow behavior during manufacturing. The results showed that mixing MES and CAPB produced a significant rise of viscosity, the addition of fatty alcohols, CMEA, and cationic polymer led to broadening of the viscosity peak only, and addition of NaCl led to a typical salt curve with a high maximum.