106th AOCS Annual Meeting and Industry Showcases

Agricultural Microscopy Division Technical Program Abstracts

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The presenter is the first author or otherwise indicated with an asterisk (*).

AM 1: Imaging and Structure

Chair: K. Koch, Northern Crops Institute, NDSU, USA

Optimization and Validation of a Rapid Method for Lipid Determination in *Nannochloropsis oculata*. L. Balduyck, C. Veryser, K. Muylaert, and I. Foubert*, KULeuven Kulak, Belgium.

Several studies have been conducted to develop a rapid method for lipid determination in microalgae, as an alternative for the time consuming gravimetric methods. Different studies showed that *in situ* lipid staining with Nile Red is a promising method, but very few did a profound optimization and validation. Therefore, two autotrophic, marine microalgae, *Nannochloropsis oculata* and *Isochrysis sp*., are selected in this study to investigate (1) whether optimization of the Nile Red assay is species dependent, and (2) whether the lipid content, determined by the Nile Red assay, could be correlated with the neutral and/or total lipid content determined by gravimetric methods.
It was found that optimization of the Nile Red assay was strongly species dependent. Consequently, optimization has to be done for each species before using the assay. For *Nannochloropsis*, a good correlation was found between total and neutral lipid content obtained by both methods.

Effect of Temperature on the Wide Angle X-ray Diffraction of Nanocrystalline Triglycerides. X. Deng[1], S. Yao[1], and G. Mazzanti[1,2], [1]Dalhousie University, Canada, [2]Institute for Research in Materials, Canada.

The peak position of Wide Angle X-ray Diffraction (WAXD) patterns of nanocrystalline triglycerides is affected by the temperature. In order to observe this effect quantitatively, pure triglycerides and triglyceride mixtures were crystallized in a desired polymorph in capillaries or in a mini Couette system. The crystallized samples were kept at different temperatures and shear rates. The detailed WAXD patterns were obtained using in-house X-ray and synchrotron X-ray sources. When the temperature increases the d-spacing for the peaks with small d-spacing increases as well. However, the d-spacing for other peaks remains unchanged or just has a very small change. The relationship between differences of d-spacing for the peaks with small d-spacing and temperature can be used to estimate the real sample temperature, especially under a high shear rate. It provides a new way to monitor and control the temperature of the system under study and the effect of high shear rate on nanocrystalline triglycerides crystallization.

PUFA Oil Oxidation Monitoring: Challenges and New Strategy Using ^{1}H NMR Technology. Z. Tan, W. Indrasena, E. Suarez, and J. Kralovec, DSM Nutritional Products, Canada.

^{1}H NMR technology has been employed for characterizing the PUFA oils and to some extent monitoring the PUFA oils oxidation. While lots of excellent work has been done by researchers around the globes, application of NMR in lipid industry is still very limited. The main objective of this study was to explore the application feasibility of this technology by critically revisiting the literature parameters and identifying the challenges with the focus on PUFA oils oxidation monitoring.

A database of ^{1}H NMR chemical shifts for a broad spectrum of PUFA oil compounds and their oxidation derivatives were built using Chemdraw and its ^{1}H NMR prediction function. Challenges for some literature parameters for PUFA oils characterizing and oxidation monitoring were identified. A new strategy for refining the parameters were proposed. Feasibility of this technology is investigated through some preliminary studies.
In summary, by refining the parameters, ^{1}H NMR technology had a very unique potential to be used for PUFA oils oxidation monitoring, especially for identifying the oxidative history of PUFA oils, thus will be very useful for optimization of the antioxidant systems and storage conditions for PUFA oil, prediction of the PUFA quality, shelf life, etc.

Development of Eco-friendly Packaging Film Using Protein Isolates. A.V. Patel[1], T.M. Panchal[1], M. Thomas[1], J.V. Patel[1], and A. Gupte[2], [1]Institute of Science and Technology for Advanced Studies and Research, India, [2]N.V. Patel College of Pure and Applied Sciences, India.

Research on biodegradable film has been in demand as the conventional plastic waste is chocking the globe. This paper focuses on fabrication and testing of biodegradable films. Protein, from maize gluten, Chitosan and Polyvinyl alcohol were the ingredients in fabricating biodegradable film amongst which protein being the key ingredient. Effect of protein concentration in resultant film was

studied using mechanical, structural, thermal, barrier and morphological behavior. Results from the study revealed that the tensile strength of film decreases gradually with increase in concentration of protein, highest of which was 22.76MPa keeping the concentration of protein optimum. Similar pattern of observation were noticed in case of thermo gravimetric analysis, elongation and water vapor transmission rate studies. Oxygen transmission rate decreases with increase in concentration of protein. All the performance testing were compared with commercially available packaging film as a standard. Results of FEG-SEM shows that all three polymers have good compatibility and disperse homogeneously. This film also shows good antimicrobial activity against model bacteria. The results reveals that protein based biofilms has great potential for packaging applications and is promising alternative for petroleum based materials.

Tween Emulsifiers Affect the Stability and Crystallization Behaviour of Partially Crystalline Oil-in-Water Emulsions. G.T. Fuller[1], T. Considine[1], M. Golding[2], A. MacGibbon[1], and L. Matia-Merino[2], [1]Fonterra Co-operative Group Limited, New Zealand, [2]Massey University, New Zealand.

Despite Tween emulsifiers being commonplace food ingredients, little comparative information is available on their functionality in partially crystalline O/W food emulsions. We studied the effect of Tween 20, 40, 60 and 80 on the shear stability and crystallization behaviour of 35wt% O/W emulsions prepared from a hydrogenated palm kernel-canola oil blend (62% SFC at 5°C) stabilized by 1wt% sodium caseinate. Onset crystallization temperature and crystallization profile were measured by pulsed NMR. The addition of 0.8wt% Tween had a marked effect on both shear stability and crystallization temperature which was highly Tween type dependent. With increasing chain length of the saturated Tweens (20, 40 and 60 respectively), shear stability increased as well as the crystallization temperature. Addition of unsaturated Tween 80 resulted in the most unstable emulsions and the lowest crystallization temperature. However, the effect of different Tween emulsifiers on the crystallization temperature was negligible in the absence of sodium caseinate. These findings indicate that alone long chain saturated Tween emulsifiers improve emulsion stability by forming robust interfacial films but with sodium caseinate may also improve stability through interfacial heterogeneous nucleation.

AM 2/EAT 4.1: Imaging Fat Crystal Networks at Different Length Scales

Chairs: K. Koch, North Dakota State University, USA; and G. Sekosan, Bunge North America, USA

Crystallization Behavior of Molecular Compound in the Binary System of 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol and 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol. K. Nakanishi[1], Y. Mikiya[1], T. Ishiguro[1], M. Sato[2], and S. Ueno[3], [1]Miyoshi Oil & Fat Co., Ltd., Japan, [2]Japan Synchrotron Radiation Research Institute, Japan, [3]Hiroshima University, Japan.

Molecular compound (MC) forms in binary system of 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (OPO) and 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (POP). It has been reported that MC forms thermodynamically stable beta form and at the 1:1 concentration ratio of OPO and POP under cooling process with cooling rate of less than 15°C/min. Industrial manufacturing process of edible oil and fat products such as margarine, however, involves rapid cooling process with cooling rate of more than 100°C/min. We revealed the crystallization behavior of MC in the binary system of OPO and POP under rapid cooling. We mixed OPO and POP at a concentration ratio of 1:1 and analyzed the mixture by changing the cooling rate from 1°C/min to 150°C/min by differential scanning calorimetry. Polymorphic analysis under slow, 5°C/min, or rapid, 40°C/min, cooling conditions was performed by using synchrotron radiation X-ray diffraction at BL19B2 of SPring-8, Hyogo, Japan.

The main results are as follows; POP and OPO crystallized individually without the formation of MC under the rapid cooling, while MC was formed under the slow cooling. In heating process subsequent to the rapid cooling process, MC was finally formed after OPO and POP individually caused polymorphic transformation into the phases with alpha or beta form.

Unpredictable Binary Systems of Triacylglycerols. P.K. Batchu[1,2], P.D. Wentzell[1], and G. Mazzanti[1,2], [1]Dalhousie University, Canada, [2]Institute for Research in Materials, Canada.

The composition, polymorphic form and proportion of the solid phases formed during crystallization affect physical properties like melting point, spreadability etc., of fat based foods. One of the steps to better understand crystallization is to be able to predict the composition and distribution of crystalline phases that would be formed from a liquid mixture of fats at any temperature. Time resolved small and wide angle x-ray diffraction patterns were obtained from crystallizing binary mixtures of trilaurin and trimyristin at different temperatures and the linear kinetic segregation (LKS) model (Los and Flöter, 1999) was tested to see if it has the capability to explain experimental phase formation. An empirical model was developed to estimate the composition and the amount of each crystalline phase formed at any point of time during the crystallization process. The (LKS) model was partially successful in explaining the formation of crystalline phases highlighting a need for improved models.

The Effects of Emulsifiers on the Formation and Morphology of Crystal Spheroids. T. Tran, A. Lim, and D. Rousseau, Ryerson University, Canada.

The effect of different emulsifiers [Span 60, Span 65, Span 80, Tween 80, glycerol monopalmitate (GMP), and glycerol monostearate (GMS)] on the shear-crystallization behavior of a model fat system [10wt% fully-hydrogenated canola oil (HCO) and 90wt% canola oil CO)] at various shear rates (400-2000 s^{-1}) was studied. Samples were crystallized between a rheometer's parallel plate geometry at 1.0°C/min which produced spheroidal crystal structures. Average crystal size decreased with increasing shear rate. The presence of emulsifiers significantly reduced crystal size ($P<0.05$) but there were no differences between the different emulsifiers. Crystal spheroid morphology was greatly dependent on the emulsifier used – crystal spheroids were formed for systems with Span 60, Span 65, Span 80, GMP, and GMS, but not for systems containing Tween 80. It was proposed that the bulky hydrophilic headgroup of Tween 80 was incompatible with the triglycerides of HCO (mostly tristearin) and hindered crystal spheroid formation. The other emulsifiers had more compatible structures that enabled them to be integrated into the spheroids' crystal matrices. In this study, we have further characterized the formation of crystal spheroids and revealed additional methods of tailoring their morphology.

Semi-empirical Treatment of Anomalous Moisture Transport into Sheared Lipids Using Magnetic Resonance Imaging. S. Paluri, M. Shavezipur, A. Abduljalil, D. Heldman, and F. Maleky, Ohio State University, USA.

This work investigates the effect of shearing on moisture diffusivity and mechanism of moisture migration in lipids. Three lipid samples: cocoa butter (CB), palm kernel oil (PO) and 20% w/w cocoa powder in palm kernel oil (CPPO) were prepared by two methods- shearing during crystallization and static crystallization. Samples' moisture uptake from a water source was measured using Magnetic Resonance Imaging and their effective diffusivity values were determined. It was observed that sheared samples had a better moisture barrier property compared to static samples. Furthermore, the mechanism of migration was found to be different between sheared and non-sheared cocoa butter samples. Regardless of processing technique, palm oil samples exhibited Fickian-diffusion controlled moisture migration. Whereas the mixture of palm oil and cocoa powder samples showed relaxation-mechanism controlled migration due to the presence of hydrophilic cocoa powder particles.

Modelling the Effects of Shear on Solid Fats Aggregation in Edible Oils. B. Townsend[1], B. Quinn[2], A. MacDonald[3], T. Gordon[4], C. Hanna[4], A.G. Marangoni[1], and D.A. Pink[2,1], [1]University of Guelph, Canada, [2]St. Francis Xavier University, Canada, [3]OneZero Software, Canada, [4]Boise State University, USA.

The aggregation of triacylglycerol (TAG) crystalline nanoplatelets (CNPs) (Acevedo & Marangoni 2010) in semi-solid fats during edible oils processing is important for determination of their functionality. Although such systems have been modeled (Pink et al 2013), they were modeled in non-flowing liquid oils. Edible oils, however, are sheared during processing. The effects of shear on the formation of solid fat structures, though highly relevant, have been unexplored theoretically. A model has now been developed to predict the aggregation structures of solid CNPs immersed in liquid oils when shear is applied. CNPs were modelled as rigid planar arrays of spheres interacting with each other and with surrounding spheres representing the liquid oil. The model was simulated using Dissipative Particle Dynamics within the software package Fluidix©. Systems of semi-solid fats without an applied shear, resulted in the self-assembly of linear stacks of CNPs (TAGwoods). Our results suggest that in the presence of shear, these TAGwoods, if formed, exhibit a distorted (non-linear) shape as suggested by observations. Larger structures are affected by shear. Predictions will be presented for outcomes of USAXS experiments. Our results might have consequences for functionality.

Self-organizing Aggregation in Complex Edible Oils. D.A. Pink[1,2], B. Quinn[1], F. Peyronel[2], and A.G. Marangoni[2], [1]St. Francis Xavier University, Canada, [2]University of Guelph, Canada.

Self-organizing aggregation structures are likely to play key roles in creating functionality in edible oils. Here we describe mesoscale models to show how different aggregation structures can arise in complex oils. We carried out modelling and computer simulations on three systems: (1) oils in which the majority solid phase is essentially-insoluble in a complex liquid phase. We considered cooling mechanisms whereby solids are 'created' at various rates which compete with the 'diffusion' processes that bring about aggregation. (2) oils in which the solid phase is significantly soluble in the liquid phase so that solid particles can dissolve and reform. Now there will be competition between characteristic solid-formation rates, solid re-structuring, and diffusion times. (3) The presence of minority components in the liquid oil of (1). Here we have different ways in which essentially-insoluble solid particles can be coated thus leading to a range of structures which suggest mechanisms via which oil binding capacity can be enhanced. We predict structures which can form together with the results of USAXS experiments. Other experiments, designed to detect key details of the model assumptions or predictions, will be proposed.

Effect of High Intensity Ultrasound on the Crystallization Behavior of Palm Oil in a Flow Cell. Y. Ye and S. Martini*, Utah State University, USA.

High intensity ultrasound (HIU, 20kHz) was used to change the crystallization behavior of palm oil in a continuous system (flow cell). Different power levels (75, 110, and 180W) and pulse duration (continuous application, 5, 10 and 15sec pulses) were used to optimize the crystallization process at 35°C. Physical properties of the crystalline network formed such as microstructure, solid fat content, melting profile, polymorphism, and viscoelasticity were measured. Results showed that the application of HIU at lower power level (75W) was the most efficient processing condition at generating higher crystallization rate which was evidenced by higher values of the Avrami

constant, *k*. The nucleation mechanism was also affect by sonication with lower Avrami indices, *n*, obtained for the sonicated samples. Higher power levels resulted in a significant increase in the temperature of the sample resulting in the melting of crystals and therefore in a delay in the crystallization. The use of HIU at low power levels (75W) in a continuous manner generated a crystalline network with higher SFC, higher elasticity, and a sharper melting profile.

Colloidal Inorganic Particle-based Edible Oleogels and Bigels. A. Patel, B. Mankoc, and K. Dewettinck, Ghent University, Belgium.

The basic building blocks which are recently being explored as structurants for oil gelation include crystalline particles (e.g. waxes), polymeric strands (e.g. cellulose derivatives) and crystalline fibres (e.g. 12 hydroxy stearic acid etc.).

In the current paper, we report for the first time, the use of colloidal inorganic particles, SiO_2 (a food-grade additive, E 551) as new structurants to fabricate clear gels in sunflower oil. Oleogels with high gel strength (G' > 10,000Pa), high resistance to flow (s_Y > 100Pa), temperature stability and a good thixotropic recovery (> 70%) were obtained at 10-15%wt of SiO_2. Cryo-SEM (along with energy-dispersive X-ray spectroscopy) revealed an interesting 'chain-like' arrangement of SiO_2 aggregates which is responsible for creating a structural framework. The fact that the positive results were only seen with hydrophilic SiO_2 (and not with functionalized hydrophobic SiO_2) strongly suggests that hydrogen bonds among surface silanol groups (Si-OH) contributed to the network formation of colloidal particles. Moreover, water phase (structured using food hydrocolloids) was added to oleogel in different proportions to obtain stable 'bigels' with interesting microstructures (as seen under cryo-SEM and confocal microscope) and excellent rheological properties.

Thermodynamic Estimates of Solid Fat Content. L. Rong[1,3], A.G. Marangoni[2], and G. Mazzanti[1,3], [1]Dalhousie University, Canada, [2]University of Guelph, Canada, [3]Institute for Research in Materials, Canada.

Food industry scientists need to predict multicomponent solid fat compositions because fat solid solutions define food texture and physical properties. Experiments were conducted with two multicomponent fats of known composition (23 and 30 Triacylglycerols) to determine solid fraction (via NMR), phase fraction, polymorphic form (via XRD) and thermal melting profile (via DSC). A database including melting temperature and enthalpy was digitized. Wesdorp's methodology, implemented in MATLAB, was used to estimate the equilibrium mole fractions of each phase. The program estimates melting temperatures and enthalpies absent from the database, and estimates interaction parameters for a 2-suffix Margules model. These parameters were used to find the composition at equilibrium for each phase. The estimated overall melting enthalpy of the mixtures was compared to the value obtained by DSC. Several predicted temperature values using Wesdorp's coefficients were unsatisfactory; hence an alternative approach was developed. The estimated overall melting enthalpies agreed well with the experimental enthalpies.

Refined Concepts on the Structures of Liquid Triacylglycerols. G. Mazzanti[1,2], L. Lin[1], R. Sanderson[1,2], O. Qatami*[1,2], and D.A. Pink[3], [1]Dalhousie University, Canada, [2]Institute for Research in Materials, Canada, [3]St. Francis Xavier University, Canada.

The structure and distribution of triglycerides (TAGs) in fats affects the properties of the final products, in terms of texture, appearance and mouth feel. A few debates and models have been developed on the distribution of TAG molecules in the liquid, but they are not consistent with the actual density of oils or our X-ray scattering (XRS) data. Four pure liquid TAG samples were examined by XRS at temperatures up to 210°C. WAXS data are consistent with the liquid phase of alkanes and other aliphatic molecules. SAXS data are similar to those produced by alcohols and fatty acids, whose molecules associate via polar groups. Therefore, we developed a new conceptual model to describe the clustering of liquid TAGs as "Loose Multimers" and from it estimated that they consist approximately of 5 to 9 molecules. The average number of molecules decreases with temperature and increases with molecular weight.