BIO 1: Biocatalysis I

Chairs: Ching Hou, USDA, ARS, NCAUR, USA; and Jun Ogawa, Kyoto University, Japan

Identification of Molecular Species of Acylglycerols of Philippine Wild Edible Mushroom, Ganoderma Lucidum Ching T. Hou*[1], Jiann-Tsyh Lin[2], Rich M. Dulay[3], and Karen Ray[4], *[1]USDA, ARS, NCAUR, USA; [2]WRRC, USDA, USA; [3]Center for R&D, Central Luzon State University, Philippines; [4]NCAUR, USDA, USA*

Wild edible mushrooms are widely consumed in many countries. Recently, we identified the molecular species of acylglycerols in the lipid extract of mushroom G. lucidum NRRL66208. One hundred and three molecular species of acylglycerols containing all normal fatty acids were identified by the MS fragmentation involved their constituent fatty acids. The chain lengths of the constituent fatty acids were from 14 to 26 carbon atoms and the numbers of double bonds were from zero to three. The contents of the molecular species of triacylglycerols in the mushroom lipid extract in decreasing order were: OOP (2.45%), OOO (1.94%), LLP (1.92%), OLP (1.80%), LLO (1.57 %), OPP (1.30%), OOL (1.09%), OOS (0.88%), and LLL (0.85%). Forty-one molecular species of acylglycerols out of 103 contained fatty acids with odd numbered carbon atoms. The contents of fatty acids with odd numbered carbon atoms in mushroom were high compared to those in higher plants. This is the first report on the molecular species of acylglycerols in mushroom.

Asymmetric Production of *Trans*-4-Hydroxy-L-Pipecolic Acid by a New Fungal Fe(II)/α-Ketoglutarate-Dependent Dioxygenase Jun Ogawa*[1], Makoto Hibi[2], Ryosuke Mori[3], Ryoma Miyake[4], Hiroshi Kawabata[4], and Satomi Takahashi[2], *[1]Kyoto University, Japan; [2]Laboratory Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Japan; [3]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan; [4]Mitsubishi Chemical Group Science and Technology Research Center, Inc., Japan*

Applications of Fe(II)/α-ketoglutarate-dependent dioxygenases (Fe/αKG-DOs) as hydroxylation biocatalysts have great advantages in regio- and stereo-selectivity. We have been found many Fe/αKG-DOs catalyzing the asymmetric hydroxylation of amino acids. L-Hydroxypipecolic acid (L-HyPip) is one of promising hydroxy amino acids valuable as building blocks for organic synthesis of various pharmaceuticals. Recently, we found a novel L-pipecolic acid (L-Pip) hydroxylase (Pip4H1) from a soil isolate, *Fusarium oxysporum* c8D. Pip4H1 catalyzes regio- and stereo-selective hydroxylation of L-Pip and produces *trans*-4-L-HyPip. Pip4H1 belongs to Fe/αKG-DO superfamily and requires α-ketoglutarate, ferrous ion, and ascorbate for its maximum activity. Five homologous enzymes (Pip4H2, 3, 6, 7 and 8) were found in a BLAST search of Pip4H1 amino acid sequence, and they also showed L-Pip *trans*-4-hydroxylation activity as well as Pip4H1. Especially, Pip4H8 derived from *Aspergillus nidulans* has wider substrate specificity than the others, and reacts with L- and D-forms of various cyclic and aliphatic amino acids. This is the first study about the fungal Fe/αKG-DO hydroxylating amino acids, and about asymmetric production of *trans*-4-L-HyPip, which is known to be constituents of several chemical compounds promising for pharmaceuticals. By using of Pip4H1 as biocatalysts, the large-scale preparation system of *trans*-4-L-HyPip becomes possible.

Recent Developments in the Production of Transgenic Oil Palm Ahmad Parveez Ghulam Kadir*, Abdul Masani Mat Yunus, Dayang Izawati Abang Masli, Bahariah Bohari, Siti Masura Subhi, Nur Hanin Ayub, Wan Nur Syuhada Wan Sulaiman, Nurfahisza Abdul Rahman, Nor Fakhrana Iskandar, Lim Fook Hwa, and Ravigadevi Sambanthamurthi, *Malaysian Palm Oil Board, Malaysia*

Production of putative transgenic oil palm using microprojectile bombardment and Agrobacterium-mediated methods has been reported using embryogenic calli or immature embryos as target tissues together with Basta as selection agent. Green fluorescent protein, mannose and 2-dioxyglucose were also evaluated. The above transformation systems, however, lack the following advantages: regenerating marker free transgenic oil palm, precise gene transfer into the nucleus and ability to transfer many minimal gene fragments into a single nucleus without having any unwanted vector backbone. These positive attributes could be achieved by transforming single cells such as protoplasts. Efforts to regenerate oil palm protoplasts were subsequently initiated using various starting materials and suspension cultures with an improved isolation protocol. These protoplasts were later subjected to 19 different combinations of plant growth regulators based on many different established as well as modified protoplast regeneration media. With the use of an agarose bead culture method, successful regeneration of oil palm protoplasts was reported. The regeneration of oil palm from protoplasts requires nearly 24 month from the time of protoplast isolation. With this breakthrough, gene transfer into oil palm protoplasts using microinjection and PEG mediated transformation was later reported. Currently, efforts towards further optimizing the transformation of oil palm using microprojectile bombardment and Agrobacterium tumefaciens, as well as improving the efficiency of transformant selection using newer selection agents and approaches, are ongoing. Some of the challenges faced and the latest efforts to establish an efficient transformation system for oil palm will be discussed.

Enhanced Alpha-galactosidase Expression in *Pseudomonas chlororaphis* Daniel K.Y. Solaiman*, Richard D. Ashby, and Nicole V. Crocker, *USDA, ARS, ERRC, USA*

Pseudomonas chlororaphis is a non-pathogenic bacterium useful for fermentative production of biopolymer (i.e., poly(hydroxyalkanoates); PHA) and biosurfactant (i.e., rhamnolipid; RhL). In order to enable P. chlororaphis to better fermentatively utilize the residual soy sugars in soy molasses – a low-cost byproduct of soybean processing, we introduced a previously cloned α-galactosidase gene of Streptomyces coelicolor (α-galSc) into the bacteria. Two approaches were employed to introduce α-galSc into P. chlororaphis. In one approach, the α-galScgene was inserted into the chromosomal DNA of P. chlororaphis (chr::gal), and the site of insertion was then mapped to ascertain its location. In the other approach, the α-galSc gene was spliced into an expression vector (i.e., pBS29-P2) to yield pBS29P2-gal which was subsequently electroporated into P. chlororaphis. Expression levels of the heterologous α-galSc in the two types of genetically modified P. chlororaphis were compared using real-time RT-qPCR technique. The results showed that both types of recombinant strains were able to transcribe the cloned α-galSc gene into its mRNA. Furthermore, the transcription level of α-galSc was many folds higher in the cells containing pBS29P2-gal plasmid in comparison to that in the cells harboring the chromosomally integrated α-galSc. This research thus yielded two active biocatalysts for future research to compare their genetic stability, α-Gal enzyme activity, and

cell growth and PHA/RhL production on soy sugars. *Pseudomonas chlororaphis* is a non-pathogenic bacterium useful for fermentative production of biopolymer (i.e., poly(hydroxyalkanoates); PHA) and biosurfactant (i.e., rhamnolipid; RhL). In order to enable *P. chlororaphis* to better fermentatively utilize the residual soy sugars in soy molasses – a low-cost byproduct of soybean processing, we introduced a previously cloned α-galactosidase gene of *Streptomyces coelicolor* (α-gal_{Sc}) into the bacteria. Two approaches were employed to introduce α-gal_{Sc} into *P. chlororaphis*. In one approach, the α-gal_{Sc} gene was inserted into the chromosomal DNA of *P. chlororaphis* (chr::gal), and the site of insertion was then mapped to ascertain its location. In the other approach, the α-gal_{Sc} gene was spliced into an expression vector (i.e., pBS29-P2) to yield pBS29P2-*gal* which was subsequently electroporated into *P. chlororaphis*. Expression levels of the heterologous α-gal_{Sc} in the two types of genetically modified *P. chlororaphis* were compared using real-time RT-qPCR technique. The results showed that both types of recombinant strains were able to transcribe the cloned α-gal_{Sc} gene into its mRNA. Furthermore, the transcription level of α-gal_{Sc} was many folds higher in the cells containing pBS29P2-*gal* plasmid in comparison to that in the cells harboring the chromosomally integrated α-gal_{Sc}. This research thus yielded two active biocatalysts for future research to compare their genetic stability, α-Gal enzyme activity, and cell growth and PHA/RhL production on soy sugars.

Production of Steryl Esters with Fatty Acids from Cottonseed Oil Using *Candida rugosa* Lipase Yuji Shimada*,*Okamura Oil Mill Co., Ltd., Japan*

Sterols and fatty acid steryl esters are known to reduce blood cholesterol level. Their physical properties, however, are different. The solubility of steryl esters in oils and fats is higher than that of sterols. The physiological and physical properties have led to development of salad oils, margarine, and mayonnaise with steryl esters and also to their use as a softener for hardened fats. In this study, fatty acid steryl esters are produced efficiently from cottonseed oil (CSO) and sterols by a one-pot two step reaction with *Candida rugosa* lipase. A mixture of CSO/sterols (2:3, by mol), 10% water, and 600 U/g lipase was agitated without dehydration, 95% CSO was hydrolyzed and the resulting FAs were esterified efficiently with sterols (esterification at the equilibrium state, 80-85%). But the reaction with dehydration reached only 60-70% esterification. The decrease of esterification was found to be due to decrease of free fatty acids (FFAs) in the reaction mixture by esterification of FFAs with glycerol produced by hydrolysis of CSO. Hence, a two-steps reaction was attempted. The first-step reaction was hydrolysis of CSO in the presence of 40% water with 30 U/g lipase. After the hydrolysis, the water layer including glycerol was removed. The second-step reaction (esterification of sterols with FFAs) was conducted with dehydration after adding 20% water, 1.5 mols of sterols for CSO, and 600 U/g lipase to the first-step reaction mixture. This one-pot two-step reaction achieved >95% esterification.

Evaluation of Selective Antibacterial Activity of Palmitoleic Acid with Co-cultivation of Stapylococcus Aureus and S. Epidermidis Toshihiro Nagao*[1], and Noriaki Kishimoto[2], *[1]Osaka Municipal Technical Research Institute, Japan; [2]Kinki University, Japan*

Atopic dermatitis (AD) is an allergy occurred by allergen, genetic factor, stress and microorganisms (skin-microbiome). Staphylococcus aureus is rarely observed in healthy control skin, and S. epidermidis is observed more than S. aureus. S. epidermidis produces several S. aureus inhibition factors. In contrast, S. aureus dramatically increases in AD skin, and the micro-organism causes considerable

aggravation of AD inflammation. Sapienic acid (SA, 6-cis-C16:1) shows selective antibacterial activity: strong activity against S. aureus and weak activity against S. epidermidis. Meanwhile, in the AD skin, the SA content decreases. So, supplementation of SA should be effective. However, SA rarely observed in natural oils. Thus, we have found that palmitoleic acid (POA, 9-cis-C16:1) observed in few natural oils showed a useful selective antibacterial activity as SA. Antibacterial activity was widely evaluated with liquid medium, but this method doesn't represent an actual skin situations. So, we developed a new method for evaluation of selective antibacterial activity of POA with co-cultivation. S. aureus NBRC13276 and S. epidermidis NBRC100911 were spread on a nutrient broth agar plate (pH6.0, 3,000cfu/cm2), and a mimic cosmetic cream including 0.001-0.25% POA was spread on the plate. After the cultivation, the cells were transferred to a mannitol salt agar plate with egg yolk. As a result, 0.03% POA in the mimic cream was effective for complete suppression of S. aureus and growth of S. epidermidis. In contrast, S. aureus was not suppressed at pH7.5 even if 0.25% POA was included in the mimic cream.

Corn Stover Hydrolysate, a Lignocellulosic Feedstock for Polyhydroxyalkanoate Biosynthesis: Property Manipulation Using a Co-feed Strategy with Levulinic Acid Richard D. Ashby*, Daniel K.Y Solaiman, and Gary Strahan, *USDA, ARS, ERRC, USA*

Lignocellulosic feedstocks are interesting materials for bio-based product synthesis because of their availability and cheap cost. Our laboratory utilized corn stover hydrolysate (CSH) as a base feedstock for bacterially-derived polyhydroxyalkanoate biopolymer synthesis. Burkholderia sacchari DSM 17165 demonstrated an ability to utilize all of the available sugars present in the hydrolysate including glucose, xylose, galactose, and arabinose to produce poly(3-hydroxybutyrate) (PHB) in yields of approximately 2 g/L and productivities greater than 40% of the dry cell weight. In contrast, Azohydromonas lata DSM 1122 only used the glucose fraction of the hydrolysate but maintained comparable yields and productivities. Using varying ratios of levulinic acid as a co-feed allowed the production of copolymers of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB-3HV; B. sacchari) or poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-4-hydroxyvalerate) (P3HB-3HV-4HV; A. lata) with varying monomer ratios ranging from 0 to 25 mol% 3HV in B. sacchari and 0 mol% 3HB to 12 mol% 3HV to 8 mol% 4HB in A. lata as determined by 1H-NMR. The number-average molecular weights (Mn) of the polymers produced by A. lata were 63% smaller than those produced by B. sacchari with comparable polydispersities (weight-average molecular weight; Mw/Mn). These physical differences provided unique polymers with controllable mechanical properties.

Concentration of PUFA in *Auranthiochytrium* sp. Single Cell Oil by Liquid Lipase Preparation Yomi Watanabe*[1], Tsunehiro Aki[2], and Araki Masuyama[3],[1]*Osaka Municipal Technical Research Institute, Japan;* [2]*Hiroshima University, Japan;* [3]*Osaka Institute of Technology, Japan*

Polyunsaturated fatty acids (PUFA) have potential benefit to human health. The world-wide increase in the consumption of fish, the conventional source of PUFA, raised the importance to develop alternative natural sources. Single cell oil from *Auranthiochytrium* sp., a marine microalgae, is uniquely rich in PUFA, especially in 22:5 (DPA) and 22:6 (DHA). In addition, the method to concentrate PUFA in oils is required for the industrial processing of pharmaceuticals and nutraceuticals. In this study, a newly developed preparation was evaluated for the purpose. First, sardine oil was used for the optimization of the enzymatic process.

Sardine oil was treated by a liquid lipase preparation kindly provided by Novozymes with 4% water at 35°C. After 22 h, 42% of hydrolysis was reached, and DHA concentration in acyl glycerol fraction was increased from 13% to 19% with the DHA recovery of 90%. The addition of ethanol to the reaction system increased the reaction degree. After 16-22 h reaction with 16-20% ethanol, nearly 80% of sardine oil was converted to FAEE and FFA, and acyl glycerol mixture containing 45-50% DHA (70-80% recovery). The condition was applied to *Auranthiochytrium* single cell oil. As its melting point was 45°C, due to the relatively high content of saturated FAs, the above reaction conditions was modified and applied to the single cell oil. The degree of reaction reached 45%, increasing DPA and DHA contents to 16 and 63% from 9% and 37% respectively, with DHA recovery of 95%, in one step enzymatic reaction.

Production of Diacylglycerol-enriched Oils by Enzymatic Interesterification and Molecular Distillation Using Soybean Oil and Distilled Saturated Monoacylglycerol Yong Wang*[1], Ying Li[2], Guanghui Li[3], Jiazi Chen[3], Qiong Chen[2], Junning Fu[2], Xiang Ma[4], and Yinglai Teng[4], *[1]Jinan University, China; [2]Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, China; [3]Dept. of Food Science and Engineering, Jinan University, Guangzhou, China; [4]Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, Guangzhou, China*

The blend of refined soybean oil and distilled saturated monoacylglycerol in a mass ratio of 70: 30 w/w were enzymatically interesterified under the optimized enzyme dosage (5% w/w) and time (5 h) at 60°C, followed by further purification through molecular distillation (MD). All samples were evaluated and compared in terms of their iodine value (IV), acylglycerols, fatty acid composition, solid fat content (SFC), crystallization and melting properties. The diacylglycerol content in the MD residual was found increased from 16.2% in interesterified oils to 22.6%. As the temperature increased, the SFC in the MD residual declined to the similar level of that in RSO. However, the crystallization onset temperature and melting point measured by differential scanning calorimetry were much higher than those of RSO. Moreover, the presence of β' crystal was found in the MD residual by X-ray diffraction and polarized light microscopy. In addition, the MD distillate had 27.4% of unsaturated fatty acids and the lowest IV amongst samples. Considering the properties above, the MD residual (diacylglycerol-enriched oil) could be considered as plastic fats for fluid margarine and the MD distillate (by-product) with 36.8 g/100 g of IV could be a potential emulsifier for oil and fat products.

BIO 1.1 / IOP 1: Biorenewable Polymers

Chairs: Richard Ashby, USDA, ARS, ERRC, USA; and Rongpeng Wang, CVC Thermoset Specialties, USA

Strategic Planning of Polymeric Materials from Vegetable Oils Zoran Petrovic*, *Pittsburg State University, USA*

Plant oils are excellent substrates for new chemistries and design of high value sustainable materials. Designing materials must take into account the structure of lipids. Essential property of materials is the cost which imposes limitation on the number of steps for conversion of oils to products. Ideally a product should be made in a single step. Direct polymerization of oils was used to prepare liquids of different viscosities useful for printing inks, plasticizers for rubber, and modifiers for asphalt etc. For better control of properties functional groups must be introduced. Functionalization of saturated lipids could be carried out by transesterification or transamidation. Unsaturated fatty acids or triglycerides are functionalized by oxidation, epoxidation, hydroformylation, metathesis, ozonolysis, thio-ene reactions with mercaptans etc. Thermosetting oil-based polymers are useful for foams, coatings, adhesives and as matrix resins for fiber-reinforced composites. They are based on multifunctional triglycerides and multicomponent curing systems. Thermoplastic polymers and elastomers can be made from two-functional fatty acids or their fragments. Excellent biodegradable shape memory materials were made from oil-based monomers. Direct polymerization of epoxidized oils leads to pure oil-based polyether foams. The largest outlet for oil-based polyols is in flexible and rigid polyurethane foams.

Sequential Liquefaction of Nicotiana Tabacum Stems Biomass by Crude Polyhydric Alcohols for the Production of Polyols and Rigid Polyurethane Foams Chiragkumar M. Patel*[1], Jina R. Patel[2], Amitkumar A. Barot[2], and Vijay K. Sinha[1], *[1]Industrial Chemistry Dept., V. P. & R. P. T. P. Science College, India; [2]V. P. & R. P. T. P. Science College, India*

In this work, Nicotiana tabacum stalks and castor oil-based polyol was synthesized via a two-step process. Preliminarily, stalks were liquefied using acid catalyst to procure glycol-glycoside and the optimized conditions for liquefaction of N. tabacum stem's biomass was 150°C temperature for 180 min time using PTSA as catalyst. Progressively, the glycol-glycoside obtained from the former step was further reacted with castor oil in the presence of lithium hydroxide to get dark brown-coloured polyol with hydroxyl value was running in between 200 and 400 IOH. Glycol-glycoside and polyols were characterized by chemical and instrumental methods. Further, by employing open-cup method involving the mixing of polyol and isocyanate adducts, the desired poly urethane rigid foam was obtained. The product was tested for their physical, mechanical, thermal, and morphological characteristics, while the thermal conductivity was in the range of 0.013 to 0.017 kcal/mh AQ1. The performed study may yield high quality rigid or semi-rigid polyurethane.

The Effect of Monoglyceride Incorporation on the Solvent Absorption and Mechanical Properties of Glycerol-based Polymer Films Prince G. Boakye*[1], Kerby C. Jones[2], Nicholas P. Latona[2], Cheng Kung Liu[2], Samuel A. Besong[3], Stephen E. Lumor[3], and Victor T. Wyatt[2], *[1]Delaware State University, USA; [2]USDA, ARS, ERRC, USA; [3]Dept. of Human Ecology, College of Agricultural Sciences, Delaware State University, USA*

Monoglycerides (MGs) have been incorporated into the matrix of poly-(glycerol-co-glutaric acid) films to investigate their effect on the thermal, mechanical, and solvent absorption properties of these films. MGs were concentrated using a combination of molecular distillation and solvent extraction, resulting in a concentrate with 99.15% purity. The MG concentrate was predominantly monoolein (92.65%). The films were made by first synthesizing polyester gels from glutaric acid and glycerol with or without the incorporation of MGs. The polymer gels were then cured at 150°C for 24 h, forming clear, solid films with a yellow hue. Solvent absorption studies revealed that poly(glycerol-co-glutaric acid-co-MG) films were able to absorb and resorb solvents better than poly(glycerol-co-glutaric acid) films, albeit they had higher erosion levels. Thermogravimetric Analysis (TGA) showed that the incorporation MGs did not remarkably affect the thermal stability of the glycerol-based films. The MG-incorporated films were qualitatively softer than the poly(glycerol-co-glycerol) films which correlates to the observed 39-fold reduction in Young's Modulus and 17-fold reduction in fracture energy. Mechanical property studies also revealed that the incorporation of MGs increased the elongation and reduced the tensile strength of poly(glycerol-co-glutaric acid) films. Correlation analysis revealed a strong linear relationship between Young's Modulus and fracture energy (R^2 = 0.9962), and between Young's Modulus and tensile strength (R^2 = 0.9972). Our study proved that MGs can be successfully incorporated in the polymer matrix of poly(glycerol-co-glutaric acid) films to produce softer films with increased elongation and increased solvent absorption capacity.

Fluorescence Emission and Catalyst Effect of Precious Metal Nanocomposites Based on Autoxidized Unsaturated Plant Oils/Fatty Acids Baki Hazer*, *Bülent Ecevit University, Turkey*

Unsaturated plant oils/fatty acids (UPOFA) have gained great interest as monomers to produce bio based polymers. UPOFA is prone to react with air oxygen under daylight at room temperature which is called "ecofriendly autoxidation". Eco-friendly autoxidation process creates peroxide linkages in order to obtain unsaturated plant oil/fatty acid polymer that can initiate the free radical copolymerization of some vinyl monomers. Mixture of salt of silver and soybean oil was spread out in a glass container and exposed to air oxygen at room temperature to obtain soybean oil nanocomposite. Similarly, a mixture of salt of gold and soybean oil was spread out in a glass container and exposed to air oxygen at room temperature to obtain soybean oil nanocomposite. Catalyst effect of gold NPs is dramatically decreased the rate of autoxidation. For example the gold catalyzed autoxidized soybean oil polymer was obtained in ten days oxidation period while the autoxidation time takes nearly one month to obtain oxidized soybean oil polymer without Au NPs. In addition, high fluorescent emission of silver/oxidized soybean oil polymer nanocomposite is obtained. The nanocomposite solutions were analyzed by UV-VIS spectrometer in view of the surface plasmon resonance. TEM was used to characterize size and shape of the metal nano particles embedded into the copolymer nano composites.

Reactivity and Structure-property Performance of Natural Oil Polyols in Polyurethanes Ibrahim Sendijarevic*, *Troy Polymers, Inc., USA*

The key principle in selection of raw materials in polyurethanes is the understanding of structure-properties relationship, as different building blocks impart different performance characteristics to polyurethane materials. In addition, raw materials are selected to meet the reactivity requirements of the applications. To meet the performance and processing requirements, in many applications it is common to use a combination of polyols. In some applications, natural oil polyols (NOPs) contribute to the performance of polyurethanes, and NOPs have been used from early days of polyurethanes industry. A perception exists that NOPs with pendant hydroxyls groups in the triglyceride backbone are less reactive compared to the conventional polyether polyols with terminal hydroxyls. However, results of recent kinetic studies demonstrated that the isocyanate reactivity of NOPs requires lower activation energy than polyether polyols, and under certain conditions NOPs are more reactive than polyether polyols. Studies of model systems also showed that the reaction profiles are not significantly affected by drop-in replacement of NOPs in place of polyether polyols in elastomers, energy-absorbing and resilient polyurethane foams. The impact of the NOPs on physico-mechanical properties of polyurethane is more significant. The effect of the NOPs on the hard/soft segment phase separation has been evaluated and correlated with dynamic and mechanical properties of model elastomers, which can be used to guide the selection of NOPs in polyurethane applications. The use of NOPs as performance materials will be demonstrated in energy-absorbing polyurethane foams.

Free Radical Polymerization of Monomers Based on Plant Oils Zoriana Demchuk[1], Kyle Kingsley[1], Oleh Shevchuk[1], Ihor Tarnavchik[1], Vasylyna Kirianchuk[2], Ananiy Kohut[2], Stanislav Voronov[2], and Andriy Voronov*[1], *[1]North Dakota State University, USA; [2]Lviv Polytechnic National University, Ukraine*

Most currently available syntheses of polymers from plant oils are limited to polycondensation and oxypolymerization. This work targets development of novel waterborne polymeric materials (latexes, dispersions, emulsions etc.) from plant oils for coatings, paints, adhesives etc. Due to highly hydrophobic nature of plant oils (triglycerides), their use for development of such materials has been challenging. To overcome hydrophobicity, converting oils into vinyl monomers to synthesize latexes via classic radical chain polymerization in emulsion was performed. Novel plant oil-based acrylic monomers are synthesized in a one-step direct transesterification (alcoholysis) of oil triglycerides. While the vinyl bond of the monomers is reactive in radical chain polymerization, the double bonds of the fatty acid fragments are unaffected and capable of post-polymerization oxidative reactions. New acrylic monomers are synthesized from soybean, linseed, sunflower and olive oil, possessing remarkably different compositions of fatty acids in triglycerides. While length of fatty acid carbon chains is similar (mostly oleic, linoleic and linolenic acids), average number of double bonds per oil triglyceride (degree of unsaturation) varies significantly. Specifically, degree of unsaturation in fatty acids was utilized as a criterion for understanding fundamental behavior of new monomers in radical chain (co)polymerization. Key questions addressed include understanding of i. how degree of unsaturation impacts new acrylic fatty monomers reactivity in (co)polymerization as well as the resulting latex properties, and ii. do polymer latexes, based on monomers from

different plant oils, demonstrate different performance in coatings, paints, adhesives, once they are copolymerized with a variety of petroleum-based counterparts?

Synthesis of a New Generation Biopolyols from Canola and Other Plant Oils Jonathan M. Curtis*[1], Tolibjon S. Omonov[2], Ereddad Kharraz[2], Xiaohua Kong[2], and M. Hossein Tavassoli-Kafrani[2], *[1]Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada; [2]University of Alberta, Canada*

Recently, various polyols have been developed from soy and other plant oils, primarily to replace petrochemical polyols used polyurethane production. In many cases these biobased polyols retain aspects of the starting triacylglycerol structure, have moderate hydroxyl numbers of 200-250 mg KOH/g and high viscosity that tends to increase significantly with increasing hydroxyl numbers and oligomeric content. Biobased polyols with high hydroxyl numbers and low viscosities are highly desirable since these can be functionally incorporated into rigid materials. In a recent patent application we described the process for the synthesis of a new class of biobased polyols from a range of unsaturated plant oils. This comprises the sequential reaction steps of (a) epoxidization of the unsaturated plant oil; (b) transesterification to produce hydroxyalkyl esters of fatty acid epoxides; and (c) further hydroxylation to obtain polyols. Using this synthetic pathway with canola oil, a polyol with high hydroxyl numbers (i.e. 350-360 mg KOH/g) and with low viscosities (i.e.

Synthesis and Characterization of Fatty Acid Modified Amines with Improved Water Barrier Properties John H. Vergara*[1], Yunze Tian[1], John J. La Scala[2], Joshua M. Sadler[2], and Giuseppe R. Palmese[1], *[1]Drexel University, USA; [2]Army Research Laboratory, USA*

Fundamental studies aimed at elucidating the key contributions to corrosion performance are needed to make progress toward effective and environmentally compliant corrosion control. Epoxy/amine systems are typically employed as barrier coatings for corrosion control, however, the curing agents used for coating applications can be very complex, making fundamental studies of water and oxygen permeability challenging to carry out. Creating model building blocks for epoxy/amine coatings is the first step in carrying out these studies. This work demonstrates the synthesis and characterization of model amine building blocks from saturated fatty acids and diethylenetriamine (DETA) with tunable hydrophobicity. The glass transition temperature (Tg) of modified amine samples suffered a 45-50 °C Tg reduction, which has been attributed to a loss of labile hydrogens available for crosslinking in these samples. It was observed that the fatty acid modified amines exhibited a reduced diffusivity to water of up to 50%. This has been attributed to the increased tortuosity of samples with a pendant aliphatic chain in the network. Samples with modified amines were observed to have lower solubility of water of up to 30%. We propose that the reduction in solubility can be caused by a dilution of oxygen in the polymer network caused by the addition of aliphatic pendant chains.

Microwave-assisted Maleation of Tung Oil for Bio-based Products Chengguo Liu[1], Zengshe Liu*[2], Brent H. Tisserat[3], Rongpeng Wang[4], Thomas Schuman[5], Yonghong Zhou[1], and Lihong Hu[1], *[1]Institute of Chemical Industry of Forestry Products, CAF, China; [2]Food and Industrial Oil Research, NCAUR, ARS/USDA, USA; [3]Function Food Research, NCAUR, ARS/USDA, USA; [4]CVC Thermoset Specialties, USA; [5]Dept. of Chemistry, Missouri University of Science and Technology, USA*

A simple, “green” and convenient chemical

modification of tung oil for maleinized tung oil (TOMA) was developed via microwave-assisted one-step maleation. This modifying process didn't involve any solvent, catalyst or initiator, but demonstrated the most efficiency of functionalizing plant oils: at a reaction time of 4 min, the yield of purified TOMA target product reached 94.5 wt.%. A mechanism of this microwave-assisted maleation was investigated by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). Moreover, three oil-based epoxides including epoxidized glycidyl ester (EGS), epoxidized soybean oil (ESO), and epoxidized octyl soyate (EOS) as well as hydroxyl-terminated polydimethylsiloxane (PDMS) were employed to react with the optimized TOMA product. Novel fully oil-based epoxy resins and silicon-containing alkyd resins were prepared. Mechanical, thermal, thermo-mechanical, and hydrophobic properties of the as-prepared epoxy and alkyd resins were evaluated.

BIO 2: Biocatalysis II

Chairs: Masashi Hosokawa, Hokkaido University, Japan; and Lu-Kwang Ju, University of Akron, USA

Enzyme-based Soy Processing Abdullah A. Loman[1], Nicholas V. Callow[1], and Lu-Kwang Ju*[2], *[1]The University of Akron, USA; [2]University of Akron, USA*

Soybeans contain three major components: protein (ca. 40%), carbohydrate (25-30%) and oil (18-20%). Soy processing ultimately should recover nutritional (and industrial) value of all these groups of components. Soy processing is currently very diverse, developed for different primary products. These diverse processes generate various byproducts and/or waste. Each requires specifically developed methods for further processing if to recover all remaining components. Developing and optimizing a series of narrowly targeted methods can be difficult and expensive. We have been developing simple, unified, enzyme-based processing to effectively recover and separate all major component groups in the soybeans. In addition, the processing allows collection of intact oleosomes and protein bodies without alteration by heat, solvent or mechanical pressing. The results obtained by this new soy processing approach will be presented and discussed.

Synthesis of Polyglycerol Fatty Acid Mono-esters by Lipase Reactions Yoshitaka Nishiyama*, Yutaro Kataoka, Hidetaka Uehara, and Yoshihiro Ueda, *The Nisshin OilliO Group, Ltd., Japan*

Polyglycerol fatty acid ester (PGE) has various features depending on its degree of esterification. In particular, monoester has a good property, such as high emulsifying or solubilizing capacity. Generally, commercial PGE is made by chemical synthesis methods, and it is known that the number of fatty acids esterified to a polyglycerol molecule varies around some central value. That is, most of the commercial PGE which is offered under the name of "monoester" has low amount of monoester in practice. Then, we approached to get a monoester-rich PGE through the use of lipase reactions. We have studied lipase reactions in oils and fats for many years, so we thought to be able to apply our know-how to synthesis of monoester-rich PGE by lipase reactions with high selectivity. First, we carried out the screening of lipases which were able to synthesize monoester-rich PGE from polyglycerols and fatty acids. Then we found that some lipases had the capability to synthesize PGE even in organic solvents, and in particular, *Candida cylindracea* lipase was able to synthesize monoester with high selectivity in *tert*-butyl alcohol. We next tried to optimize some reaction conditions to increase the reaction rate. Then we found that initial water content in the reaction system significantly affected the reaction rate. Consequently, we were able to acquire the high-purity polyglycerol fatty acid monoester in a relatively short reaction time.

Restructuring Lipids Enzymatically Casimir C. Akoh*, *University of Georgia, USA*

Lipases are useful biocatalysts in lipid modifications to add value, produce functional, healthful and nutraceutical lipids. Intake of high amounts of trans fatty acids (TFAs) have been positively correlated with increased risk of several chronic diseases and is being phased out. Alternative technologies and products to replace trans fat are currently of interest. Structured lipids (SLs) lipids can be prepared to replace the functionality of trans fats in margarines, shortenings, and spreads formulations. Palm and palm kernel oils as well as stearic acid-containing fats are often part of the substrates. Breast feeding

has long been accepted as the best practice for infant feeding. However, as better understanding of the nutritional needs of the infant emerges, it is possible to target these needs using specific SLs. Structured lipids as infant formula fat analogs that mimic breast milk fatty acid composition were synthesized using enzymes. These SLs resembled human milk fat (HMF) while containing functional and physiologically important fatty acids for infant health. Our research resulted in the production of trans-free SLs as an alternative to partially hydrogenated fat and can be used by the food industry to formulate trans-free foods. The SLs have high sn-2 palmitic acid content and comparable fatty acid composition to HMF.

Preparation of Phytosteryl Ester and Enrichment of Stearidonic Acid via One-step Lipase-catalyzed Esterification Nakyung Choi[1], and In-Hwan Kim*[2], *[1]Korea University, South Korea; [2]Korea University, Republic of Korea*

Synthesis of phytosteryl ester and enrichment of stearidonic acid were carried out simultaneously with phytosterol and fatty acid from Ahiflower® seed oil via one-step lipase-catalyzed esterification. A commercial lipase (Lipase OF) from *Candida rugosa* was employed as a biocatalyst. Fatty acid residues in Ahiflower® seed oil was composed of 5.23 mol% palmitic acid, 1.76 mol% stearic acid, 9.43 mol% oleic acid, 12.41 mol% linoleic acid, 5.77 mol% γ-linolenic acid, 45.94 mol% α-linolenic acid, and 19.47 mol% stearidonic acid. Four solvents were screened and cyclohexane was selected as the best solvent for reaction medium. The parameters investigated were enzyme loading, temperature, and amount of solvent. The optimum enzyme loading, temperature and amount of solvent were 10%, 30°C, and 2 mL/g substrates, respectively. The conversions as well as the enrichment of SDA were improved by addition of molecular sieve after 1 h of reaction. Consequently, under the best condition including addition of molecular sieve, the maximum conversion and SDA content in residual fatty acid were 75% and 57 mol%, respectively.

Improving the Positional Specificity and the Reaction Efficiency of Phospholipase D-mediated Phosphatidylinositol Synthesis Yugo Iwasaki*[1], Jasmina Damnjanovic[2], Michiko Muraki[2], and Hideo Nakano[2], *[1]Graduate School of Bioagricultural Sciences, Nagoya University, Japan; [2]Nagoya University, Japan*

Phospholipase D (PLD)-catalyzed transphosphatidylation can be used for the synthesis of various phospholipids. Commercially available, wild-type *Streptomyces* PLD cannot synthesize phosphatidylinositol (PI), but we have created mutant PLD variants having PI-synthesizing activity. One of the PLD variants (NYR variant) showed positional preference towards the 1- and 3-hydroxyl groups of *myo*-inositol, giving 1-PI and 3-PI at the ratio of 76/24. The purpose of this study is to improve the positional specificity of the NYR variant, so as to synthesize exclusively pure 1-PI, a natural PI isomer. First, a saturation mutagenesis was performed at five amino acid residues in the enzyme's substrate-binding pocket, making 95 variants. The variant enzymes were prepared using a recombinant expression system, and the positional specificity was evaluated. As a result, we identified a variant NYR-186T as the most improved one, which showed 1-PI/3-PI ratio of 93/7 at 37°C. Lowering the reaction temperature further improved the specificity to 1-PI /3-PI ratio of 97/3 at 20°C. Next, we tried to improve the PI yield by optimizing the enzyme reaction conditions. We found that, in the water-solvent biphasic reaction system, addition of high concentration of NaCl in the aqueous phase enhances the reaction efficiency to several fold. Under the optimized conditions, the PI content reached ~40%. Finally,

with this improved system, we could successfully synthesize and isolate some PIs with defined chemical structures, namely 18:1/18:1-PI, 16:0/18:1-PI, 18:0/20:4-PI and 18:0/22:6-PI, with overall yields of 25~37%, and PI isomeric purities of 91~96%. References: Damnjanović,*et al, Biotechnol. Bioeng.,* **113**, 62-71 (2016). Muraki *et al, J. Biosci. Bioeng.,* **122**, 276-282 (2016).

Metabolism and Beneficial Function of Docosapentaenoic Acid Masashi Hosokawa*[1], Yanzhu Yanzhu Tian[1], Kazuo Miyashita[1], Donato Romanazzi[2], and Tadahiro Tsushima[3], *[1]Hokkaido University, Japan; [2]Cawthron Institute, New Zealand; [3]Bizen Chemical C. Co. Ltd., Japan*

Docosapentaenoic acids (DPAs) are long chain polyunsaturated fatty acids which are found in several seafoods and marine microbial oil. n-3 DPA was converted to docosahexaenoic acid in human hepatoblastoma HepG2 cells and murine macrophage-like cell, RAW264.7. In addition, retro-conversion to EPA was also observed in HepG2, RAW 264.7 cells, and human colon carcinoma Caco-2 cells. The retro-conversion of n-3 DPA led to a greater increase of EPA in the phospholipid fraction than in the neutral lipid fraction. Furthermore, n-3 DPA suppressed mRNA expression of pro-inflammatory cytokines in activated RAW264.7 cells. The suppressive effect of n-3 DPA was stronger than that of EPA and the same level as DHA. In vivo study, DPA-rich lipid decreased serum cholesterol and glucose concentration in the diabetic /obese KK-Ay mice, which express hyperlipidemia and hyperglycemia. These data show that n-3 DPA is a functional fatty acid with unique bioconversion and beneficial effects.

Regulation of Carotenoid Biosynthesis in Marine Thraustochytrid, *Aurantiochytrium* sp. Kenshi Watanabe, Hirokazu Takahashi, Yoshiko Okamura, Takahisa Tajima, Yukihiko Matsumura, Yutaka Nakashimada, and Tsunehiro Aki*, *Hiroshima University, Japan*

Carotenoids such as astaxanthin and canthaxanthin are the pigments having some conjugated double bonds derived from isoprenoid backbone. They show a high antioxidant activity and therefore have attracted attention from the markets of functional foods, cosmetics and culture feeds. Preexisting sources of these carotenoids however involve the problems of safety and production cost. The marine thraustochytrid, *Aurantiochytrium* sp., producing polyunsaturated fatty acids are also known to accumulates the antioxidative carotenoids in the cells, being expected as an industrial producer. We have isolated some thraustochytrid strains producing significant amounts of the antioxidative carotenoids and analyzed their profiles of carotenoid production under a various culture conditions. The structure-function relationship and the expressional regulation of enzymes participating in the carotenoid biosynthesis, especially a multi-functional carotene synthase, have also been studied. Recent progress of these topics will be reviewed.

Novel Conjugated PUFAs Produced by Anaerobic Bacteria via the Biohydrogenation of C20 PUFAs Shigenobu Kishino*, Kousuke Mihara, and Jun Ogawa, *Kyoto University, Japan*

Conjugated fatty acids have attracted much attention as a novel type of biologically beneficial functional lipid. Previously, we found that Lactobacillus plantarum can convert linoleic acid into conjugated linoleic acid (CLA) and identified three enzymes for CLA production. However, this enzyme system couldn't convert C20

polyunsaturated fatty acids (PUFA) such as arachidonic acid (5Z,8Z,11Z,14Z-20:4, AA) and eicosapentaenoic acid (5Z,8Z,11Z,14Z,17Z-20:5, EPA) into corresponding conjugated fatty acids. Recently, conjugated eicosapentaenoic acids (CEPAs) and conjugated arachidonic acids (CAAs), produced by alkali-isomerization, were reported to show the potent cytotoxicity against several cancer cells. Therefore, CAAs and CEPAs are expected to be a promising as safe anti-cancer agents. In this study, we screened the anaerobic bacteria possessing the PUFA-conversion activity and found that Clostridium bifermentans JCM 1386 hydrogenated AA and EPA into 5Z,8Z,13E-20:3 and 5Z,8Z,13E,17Z-20:4, respectively. Furthermore, through further analysis, we revealed that conjugated fatty acids were intermediates of the PUFA-biohydrogenation by the cell-free extracts of this strain, and succeeded in obtaining the novel CAAs and CEPAs. Finally, this strain can convert PUFA with cis-omega6,cis-omega9 double bonds such as linoleic acid, linolenic acid, stearidonic acid, dihomo-gamma-linolenic acid, AA, and EPA into two corresponding conjugated fatty acids with trans-omega7,cis-omega9 and trans-omega7,trans-omega9 double bonds.

BIO 2.1 / IOP 2: Biofuels

Chairs: Adeeb Hayyan, University of Malaya, Malaysia; Lieve Laurens, National Renewable Energy Laboratory, USA; and Jun Ogawa, Kyoto University, Japan

Recovery of Fatty Acids from Advanced Biofuels: Improvement in Acid Number and Value Justice Asomaning*, and David C. Bressler, *University of Alberta, Canada*

The development of renewable alternatives to fossil derived chemicals and fuels haveattracted a significant interest due to socioeconomic and environmental concerns. Advanced biofuels, particularly from lipids that can be used as drop-in fuels, which is directly compatible with current petroleum infrastructure has seen considerable advancements over the last couple of years. One of the major drawbacks of advanced biofuels is the high acid number as a result of residual acids, which necessitates further processing to convert the residual acids hydrocarbons using techniques such as hydrotreating. The aim of the present study was to develop methods for removing and recovering fatty acids from advanced biofuels thereby reducing the acid number to acceptable level. The recovered fatty acids can then be utilized in high value application such as in the cosmetic and pharmaceutical industries. Various methods were used to directly remove the acids from the advanced biofuels. The systems were then tested for their ability to recover the acids and also regenerate the system. Results show that the acids can be successfully removed from advanced biofuels using these methods providing acid numbers below detectable levels. The systems were then used for ten cycles after regeneration without significant loss in acids removal from the advanced biofuel. This study demonstrated the feasibility of improving the quality and value of advanced biofuel through acid number reduction and fatty acids recovery.

Synthesis and Purification of Polyphenolic Branched-chain Fatty Acids with Natural Monophenols Helen Ngo Lew*[1], Zongcheng Yan[2], Karen Wagner[1], and Robert A. Moreau[1], *[1]USDA, ARS, ERRC, USA; [2]South China University of Technology, China*

Polyphenolic branched-chain fatty acids (poly-PBC-FAs) are hybrid compounds produced by linking electron-rich aromatic rings such as natural monophenols (i.e., thymol, carvacrol and creosote) with mixed free fatty acids derived from soybean and safflower oils through a process known as arylation. The antimicrobial properties of these poly-PBC-FAs are similar or better than their parent monophenol compounds. This paper will focus on the continuing development of the arylation process using mixed fatty acids coupled individually with thymol, creosote, and carvacrol in the final products to give poly-PBC-FA yields of 72.4%, 77.2%, and 48.8%, respectively. The difference in yields is strongly dependent on the different functional groups on the three phenolic aromatic rings. Water is a co-catalyst in the reaction and the concentration of water has a significant influence on the arylation reaction by depressing the isomerization reaction. Another aspect of this presentation will focus on the development of a highly efficient distillation method to clean up the products achieving up to 97% purity, which is extremely important for evaluating their antimicrobial properties.

Animal Fatty Wastewater Sludge recovery by Acid-catalyzed Esterification into Fatty Acid Butyl Esters as Potential Biodiesel Christopher Wallis[1], Muriel Cerny[1], Eric Lacroux*[2], and Zéphirin Mouloungui[1], *[1]Laboratoire de Chimie Agro-Industrielle, France; [2]Chimie Agro-Industrielle, France*

Animal Fatty Wastewater Sludge (AFWS) represent a novel source of glyceride and free fatty acids (FFA) material for the future. These lost lipids were studied for potential conversion into biodiesel. Often referred to floatation greases, AFWS were found to be composed of 20-70% of water, demonstrating the variability in composition that one can expect from such slaughterhouse wastes. Generally regarded to be completely hydrolyzed to FFAs, however, as our analysis shows they contain up to 20 % of tri-, di- and mono-glycerides. Whilst current technologies employed for biodiesel production are very effective when applied to clean sources of lipids (trans-esterification of glycerides; FFAs esterification), they are far less effective when applied to mixtures, or if there is significant amount of water present. Fatty acids esters were synthesized in complex aqueous media. The 4-dodecylbenzenesulfonic acid catalyzed esterification of AFWS with 1-butanol was performed in a novel batch reactor fitted with a drying chimney for “in situ” water removal. Conversion yield was optimized using a Doehlert surface response methodology. Two products that meet the biodiesel standards (Biodiesel European Standard EN14214): First type of product developed with properties similar to diesel, Fatty Acid Butyl Esters (FABEs) were isolated in good yields (analysed on CG-FID: 95 %+). The second original biodiesel developed is a blend of FABEs with 1-butanol (16%). These two potential biofuels were analyzed in comparison with current analogous biofuels (FAME based biodiesel, FABE products from vegetable oils) and were found to exhibit high cetane numbers and flash point values.

Ionic Liquids Derived from Amino Acids for Catalytic Biodiesel Production Jingbo Li* and Zheng Guo, *Aarhus University, Denmark*

Ionic liquids (ILs) derived from biomolecules have attracted more and more attention due to their generally biodegradable, non-toxic, and more sustainable properties. Amino acids are a group of biomolecules with additional unique property that they are able to be converted into both cations and anions. Herein, we synthesized a series of amino acid-based ionic liquids (AAILs) possess either strong acidic or strong basic properties. Structure evolution strategy was adopted to gain the most promising AAILs for catalyzing biodiesel production. The strong acidic AAILs were used to catalyze esterification reaction to convert free fatty acids into biodiesel while the strong basic AAILs were employed to catalyze transesterification reaction to convert acylglycerides into biodiesel. The catalytic activity of AAILs was strongly correlated to the properties of the side chains of the parental amino acids. [Asp+][NO_3-] and [Choline+][Arg-] were the most effective for esterification and transesterification, respectively due to the strong acidity and basicity of the side chains of aspartic acid and arginine. The best catalyst, [Tetrabutylammonium+][Arg-], was obtained following structure evolution strategy. Esterification efficiency of 97% was achieved within 5 h, at 70°C, with 10% [Asp+][NO_3-] loading and 7.5:1 methanol to oleic acid ratio. Complete transesterification of triglycerides was achieved within 15 min, at 90°C, with 6% [Tetrabutylammonium+][Arg-] loading and 9:1 methanol to triglyceride ratio without soap formation. The AAILs directly used as catalysts for both esterification and transesterification reactions may result in greener and sustainable biodiesel

production processes in the future, which highlights the importance of the current study.

Grease Formulation Using Post-consumed Clothes: A Sustainable Approach Amitkumar A. Barot*[1], Chiragkumar M. Patel[2], Tirth M. Panchal[3], Jigar V. Patel[3], and Vijay K. Sinha[2], *[1]V. P. & R. P. T. P. Science College, India; [2]Industrial Chemistry Dept., V. P. & R. P. T. P. Science College, India; [3]Dept. of Industrial Chemistry, Institute of Science and Technology for Advanced Studies and Research, India*

Recent scenario demands environmentally adaptable routes for recycling synthetic polymeric waste. Also, the demand of bio-based products is increasing due to strict environmental laws. Finding a suitable way to recycle polyester is a worldwide concern due to its environmental impact and increasing volume of these materials produced in society. In the field of lubricants, much effort has been spent on substitution of petro-based raw materials by natural-based renewable ones. In this frame work, current paper shows the utilization of castor oil as replacement to diols in recycling of polyester waste. This in comparison with diols is renewable, easily available, environmental friendly, economically cheaper and hence sustainable indeed. Glycolyzed products were then used as base oil in the formulation of grease. Glycolyzed products were characterized by using various chemical and instrumental methods and prepared greases were evaluated for its tribological properties.

Enzymatic Catalyzed Fat-splitting as Replacement to Chemical Fat-splitting Process Anders Rancke-Madsen, Hans Christian Holm, Per Munk Nielsen, and Simon Emil Lausen*, *Novozymes Denmark*

Conventional chemical fat-splitting processes suffer from environmental problems and equipment corrosion. Enzyme-catalyzed fat-splitting is an attractive alternative and lipases offer many advantages such as low CAPEX, high quality and environmental safety. However, enzymatic processes are only applied to a limited extent at industrial scale due to low degrees of conversion, typically < 95%, and relatively high costs of current fat-splitting lipases. A protein engineered variant of Thermomyces lanoginosus lipase expressed in high yields has been tested at 55oC/131oF in 1L-stirred glass reactor system using castor oil as feed-stock, a two-step reaction set-up, 100% water w/w and 0.6% w/w enzyme solution. The results suggest that the new lipase is superior to traditional lipases and gives both higher degree of conversion and lower costs. Kinetic studies and initial engineering assessments of industrial scale applications suggest that enzymatic catalyzed fat-splitting technology holds a great potential to replace chemical fat-splitting process within a few years.

Process Development of a Sustainable Aromatic Hydrocarbons Derived from *Camelina sativa* Randy L. Maglinao*[1], Chazley J. Hulett[2], Eleazer P. Resurreccion[2], and Alexandra K. Jones[1], *[1]Advanced Fuel Center, Montana State University-Northern, USA; [2]Montana State University Northern, USA*

Aromatic compounds in petroleum-based jet fuels are necessary to give the fuel's desirable lubricity and seal swelling properties. Without these compounds in jet fuel, operational problems, such as failure of fuel tank gaskets, could occur and with disastrous consequences. Research on the synthesis of renewable aromatics mostly focused on pyrolysis of lignin coupled with hydrotreating. We report an alternative feedstock and synthesis route for selectively producing renewable aromatic hydrocarbons, which can be used in both as a blend component for jet fuel. Using fatty acid methyl esters derived from camelina, renewable aromatics and straight chain hydrocarbons were

produced through a two-step process. Batch reactor systems (20 – 30 mL) were used to convert FAME to hydrocarbons. Results showed that the polyunsaturated fatty acids in camelina FAME yield renewable aromatics. It is also found that multisubstituted benzenes were produced.

Novel Building Blocks Designed from Metathesized Vegetable Oils Frederyk Ngantung*, *Elevance, USA*

Elevance produces high-performance, cost-advantaged and bio-based chemicals from metathesized vegetable oils. The processes use a highly efficient, selective catalyst to break down natural oils such as soybean or palm oil and recombine fragments. The resulting products are high-value, difunctional chemicals with superior functional attributes previously unavailable commercially. These chemicals can be used as ingredients and building blocks for detergents, personal care, oil field and agriculture chemicals. In this talk, two industrial examples will be presented to showcase the applicability of Elevance's ingredients and building blocks. Example 1. Hydraulic fracturing chemicals D-limonene when added as fracking fluid additive has been demonstrated to improve production rate of gas in many conventional and unconventional reservoirs and basin. The availability and price of d-limonene, however, have been volatile in the past five-to-six years. Elevance HFS™ is a high-performing, hydraulic fracturing fluid ingredient that can be a cost-effective substitute to d-limonene. In independent laboratory tests where Elevance HFS™ was blended into hydraulic fracturing fluids and compared to d-limonene- based fluid, Elevance HFS™ clearly outperformed the d-limonene-based fluids in enhancing the productivity of oil and gas wells. Example 2. Building blocks for surfactants in laundry detergents A series of methyl ester ethoxylates were prepared from our building block. Their physical properties and efficacy in fabric care were evaluated and compared to incumbents. It was found that the unsaturated methyl ester ethoxylates derived from Elevance Inherent building blocks exhibited superior cleaning performance, foam control, viscosity control and more compact formulations when compared to standard ethoxylates.

Sterol Molecular Fingerprinting in Different Algae Provide Options for High-value Co-Product Development in a Conversion Process Lieve Laurens*, Oliver Palardy, Keegan Duff, and Stefanie Van Wychen, *National Renewable Energy Laboratory, USA*

Improving biomass production and utilization is imperative for commercializing a future algae-based biorefinery. Sterols are high-value products that can be isolated from algal biomass and have not been studied before in the context of a conversion pathway to biofuels and bioproducts and provide value as either nutraceuticals or as feedstocks for novel surfactant production. Mass spectral analysis indicates that there are 11 sterol compounds in Chlorella and 16 in Scenedesmus. The compounds we identified fall within two major classes: 28-carbon 4-desmethylsterols and 29-carbon 4-desmethylsterols. Within the 28-carbon group, the spectra either more closely resemble campesterol (MW 472) or ergosterol (MW 468). Within the 29-carbon group, the spectra could be grouped into compounds with a MW of 484 (EG stigmasterol, fucosterol, Δ5-avenasterol) and those with a molecular weight of 486 (sitosterol or Δ7-stigmastenol). The commercially available surfactants (e.g. linear alkyl benzene sulfonates) are often mixtures of compounds. We demonstrate here that the variety of sterols present is greater in Scenedesmus than in Chlorella, which seems to be almost entirely 28-carbon variants of ergosterol, while the former contains a mixture of both 28 and

29-carbon sterols. We present data on a new method for sterol characterization in algae and highlight the growth phase specific accumulation of this class of compounds along with preliminary data on conversion of sterols to non-ionic surfactants, and surfactant characterization after esterification with succinic anhydride and polyethylene glycol.

Determination of Solubility and Kinetic Parameters for Switchable Solvents Using Microfluidics Ghata M. Nirmal*[1], Thomas F. Leary[2], and Arun Ramchandran[2], *[1]University of Toronto, St. George, Canada; [2]University of Toronto, Canada*

Current separation processes in the industry involve use of specific solvents at every step, which can be material and energy intensive. An alternative, eco-friendly route is utilization of CO_2-triggered switchable solvents. These solvents are capable of switching properties, such as hydrophilicity, polarity or ionic strength, upon reaction with carbon dioxide. They revert their property when heated, or in the presence of an inert. Application of these solvents in the current industrial setup entails optimization of operational parameters, for which we use microfluidics. The experiments are carried out in the Taylor flow regime, which involves alternating segments of gas and liquid flowing in a straight circular capillary. The advantage of this setup is the availability of high interfacial areas and well-characterized flow profiles. As the mixture flows through the capillary, the gas segments shrink in size. We provide a systematic framework to interpret this data to extract solubility and kinetic parameters (e.g. diffusivity, reaction rates), supported by scaling analysis and simulations. To demonstrate our experimental method and analysis approach, experiments were carried out in circular, silica capillaries of different radii by generating segmented flow of CO_2 in physical solvents such as acetonitrile and propylene carbonate. Our approach can be applied to diverse gas-liquid reaction systems to determine optimum operational parameters, and thereby moving a step closer towards implementation of green chemistry in the industrial setting.

BIO 3: Advances in Bioactive Fats

Chairs: Suk Hoo Yoon, Woosuk University, Korea; and Masashi Hosokawa, Hokkaido University, Japan

Effects of Heating Methods on Thermal Isomerization and Degradation of Carotenes Suk Hoo Yoon*, *Woosuk University, Korea*

Thermal isomerization and degradation of ß-carotenes were carried out in air and in triacylglycerols. Carotenes in triacylglycerols were degraded faster than that in air. The isomers produced with and without oxygen identified were 15-cis-, 13-cis-, and 9-cis- ß-carotene. The presence of oxygen accelerated the isomerization of all-trans-ß-carotene more rapidly in the initial heating period than that in absence of oxygen. After 30 min heating, more than 90% of trans-ß-carotene and its isomers were destroyed with and without oxygen. Amounts of trans-ß-carotenes and all-trans-α-carotenes in pumpkin decreased with an increase in heating time. The proportion of 13-cis-ß-carotene increased after heat treatment started, and heating methods did not change the proportions of isomers.

Synthesis of Trimethylolpropane Esters Using an Immobilized Lipase Heejin Kim*[1], and In-Hwan Kim[2], *[1]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea; [2]Korea University, Republic of Korea*

Synthetic oleochemical esters of polyols and fatty acids are biodegradable and possess desirable technical and ecological properties. In particular, trimethylolpropane (TMP) esters have been applied widely as hydraulic fluids. In this study, TMP triester was successfully synthesized by lipase-catalyzed esterification with TMP and high oleic palm fatty acids using a newly immobilized lipase. The immobilized lipase was prepared with liquid Lipozyme TL 100 L from *Thermomyces lanuginosa* using Duolite A568 as carrier. The effects of temperature, enzyme loading, and vacuum on the synthesis of TMP triester as a function of reaction time were studied. The optimum temperature, enzyme loading, and vacuum were 50°C, 10% (based on the total weight of the substrate), and 0.7 kPa, respectively. Under the optimum conditions, the maximum yield of TMP triester reached up to 97% after 24h. A recirculating packed bed reactor (RPBR) system was also employed for efficient production of TMP esters using the immobilized lipase. The maximum TMP triester of 95% was obtained during 24 h at the optimum conditions in RPBR. Lubrication properties such as viscosity, viscosity index, pour point and cloud point of synthesized TMP esters were also determined.

Functional Lipid Production by Microalgae Phaeodactylum Tricornutum Yu-Hong Yang[1], Lei Du[1], Masashi Hosokawa[1], Kazuo Miyashita*[1], Noritaka Yoshikawa[2], Yume Kokubun[2], Hisayoshi Arai[2], and Hiroyuki Taroda[2], *[1]Hokkaido University, Japan; [2]DIC Corporation, Japan*

Marine diatoms make up an important group particularly for biogeochemical cycling of carbon. They contribute to approximately 40% of primary productivity in marine ecosystems and 20% of global carbon fixation. Due to the high productivity of organic compounds, diatoms such as Phaeodactylum tricornutum and Thalassiosira pseudonana have been explored as sources of bioactive metabolites. For example, the diatom has ability to synthesize lipid as a storage compound at 20%–30% of dry weight. In the present study, we report the functional lipid production by Phaeodactylum tricornutum. P. tricornutum was cultivated in a 100 mL media, and then, the

cultivation was scaled up to 70 L in stages. The cultures were aerated by constant CO_2 gas bubbling at 20ºC. After extraction of the total lipids (TL) from dried P. tricornutum, the TL was separated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL). P. tricornutum contained 321.89 mg/g (dry weight) total lipids and 4.47 mg/g (dry weight) fucoxanthin. NL was the major constituents of P. tricornutum lipids, in which triacylglycerol was the predominant component. GL contained 1.66% sulphoquinovosyl diacylglycerol, which contained a high percentage of eicosapentaenoic acid (20:5n-3, EPA) (32.8%). Phosphatidylcholine and phosphatidylglycerol were the major component of PL. EPA was found to be distributed in all lipid fractions. Besides, hexadecanoic acid (C16:0) and palmitoleic acid (C16:1) were the principal fatty acids in most lipid classes.

Effects of Peanut Oil on Regioselectivity Yarrowia Lipolytica Lipase in Hydrolysis Reaction Emília Akil*[1], Priscilla Amaral[2], Jerôme Lecomte[3], Torres Alexandre[2], and Pierre Villeneuve[4], *[1]Federal University of Rio de Janeiro, Brazil; [2]UFRJ, Brazil; [3]CIRAD, Greece; [4]CIRAD/INRA, UMR 1208 IATE, France*

Lipases are able to catalyze the hydrolysis of the ester bond of tri-, di- and mono-glycerides of long-chain fatty acids (FAs) into FAs and glycerol. The applications of lipase are intimately related to their regioselectivity, which is the ability to distinguish the two external positions of the triacylglycerol (TAG) backbone or the internal position. Previous studies showed that Y. lipolytica lipase is a strictly 1.3-regioselectivity in some lipids in few minutes of hydrolysis. Peanut oil is the food of interest because it can inhibits pancreatic lipase and reduce hypoglycemic and hypolipidemic effects. This work aimed at investigating the regioselectivity profile of extracellular free lipase from Yarrowia lipolytica 583 (IMUFRJ 50682) in peanut oil in hydrolysis, produced by submerged fermentation. The performance of Y. lipolytica lipase in TAG from peanut oil was peculiar, which in lipids classes values generated by hydrolysis showed irregular variations and higher standard deviation. Same behavior is observed for 1.2/2.3- and 1.3-diacylglicerol values. All polar compounds were removed from the peanut oil and a great behavior was observed, consequently, a strong 1.3-regioselectivity profile. Polar compounds from peanut, such as phenolic compound and diacylglicerols distribution, could be inhibitors of pancreatic lipase. As Y. lipolytica lipase shows the same profile as pancreatic lipase, strictly 1.3-regioselectivity in lipids, probably these polar compounds inhibited lipase specificity, affecting its performance. These new results from free Y. lipolytica lipase allow greater knowledge from this lipase, once it has a strong 1.3-regiospecific in hydrolysis reaction, although for peanut oil it shows a completely different behavior.

Pennycress—A Novel Emerging Oilseed Providing a Unique Oil Feedstock for Food and Biofuels Tim Ulmasov*, *Arvegenix, USA*

Pennycress is a winter cover crop protecting the soil from erosion, preventing the loss of nitrogen to the water systems, and helping hold nutrients and residues to improve soil productivity. While it is well established that cover crops provide agronomic and ecological benefits to agriculture and environment, only 5% of farmers today are using them. The reason is purely economical – it requires on average ~$30/acre to grow a cover crop on the land that is staying idle between two seasons of cash crops such as corn and soy. Arvegenix has joined efforts with researchers in academia to develop an economically viable crop that will provide a competitive solution to the sustainable agriculture problem and generate

additional income for farmers instead of an out-of-pocket investment. Field Pennycress is a winter annual belonging to the Brassica (mustard) family. It is a close relative of Arabidopsis with a small diploid genome, allowing one to apply tremendous knowledge and power of Arabidopsis genetics for domesticating this new crop. Pennycress seeds are much bigger than Arabidopsis and typically contain 36% oil content, roughly twice the level as a soybean, with a super low saturated fat content (<3.5%). Aggressive breeding and gene editing research programs are underway generating commercial lines that emerge consistently, produce high yields, and contain oil and meal properties that are ideal for food and industrial oil applications, commercial biofuels, and animal feed. The target growth area is in the Midwest with ~35M acres bare land opportunity for planting between corn and soy.

BIO 3.1 / PRO 3.1: Biodiesel from Low-quality Feed Stocks

Chairs: Casimir Akoh, University of Georgia, USA; and Per Munk Nielsen, Novozymes, Denmark

Lipase-mediated Biodiesel Production and Its Commercialization Progress Dehua Liu*, *Tsinghua University, China*

Although it is well recognized that enzymatic process has tremendous advantages versus alkali/acid-based catalytic process, the low stability (poor operational life) and the high cost of the lipase have been the main hurdle to the industrialization of lipase-catalyzed biodiesel production. Tsinghua University has been engaged in enzymatic process for biodiesel production for more than 10 years and great breakthrough technologies/equipments including integrated use of different lipases, on-line water removal, membrane technology for recovering lipase as well as development of a novel air-lift bioreactor have been developed/achieved successfully, with which the lipase's operational life is improved greatly, leading to significant reduction in lipase cost. This enzyme-mediated biodiesel production has been commercialized with a capacity of 50,000 tons/y biodiesel in China and a bigger one (200,000ton/year) is being under construction. This process has also been demonstrated successfully in Brazil. The successful running of this technology is attracting attention worldwide, and companies from the United States and European countries are exploring the potential of licensing this technology for large-scale biodiesel production based on low quality oil feedstocks. In 2016, a biodiesel plant (with a capacity of 50,000 tons/y) from Texas, US decided to use this enzymatic process for biodiesel production (the company originally use alkali-based catalysis) and currently they are modifying the related equipment and shifting the production from alkali-based process to lipase-mediated process.

Industrial Applications in Continuous Enzymatic Biodiesel Processing Brent Chrabas* and Stu Lamb, *Viesel Fuel LLC, USA*

The ability to optimize process inputs in a continuous system has lead to the development of the Continuous Enzymatic Biodiesel Process. Working in collaboration with Novozymes, Viesel Fuel, LLC is pioneering the use of enzymes in their Continuous Enzymatic Biodiesel Process in Ft Myers, Florida. Initially brought to pilot scale out of the laboratory in 2014, the construction of the full scale Continuous Enzymatic Biodiesel Process was completed in 2016. The Industrial Application of the Continuous Enzymatic Biodiesel Process being demonstrated by Viesel Fuel, LLC at its South West Florida production facility was retrofitted from an idled biodiesel plant which utilized traditional chemical catalysts and has a nameplate capacity of 9 MMGY. The laboratory capabilities and process controls required to support production will be highlighted as the general process flow of the Continuous Enzymatic Biodiesel Process is outlined in this presentation.

Biodiesel Produced from Oil Recovered from Waste Water Plants Frankie Mathis*,*Tactical Fabrication LLC, USA*

Tactical Fabrication has in the past years been gathering data on unit operations to clean oily waste (Fats, Oils and Grease/FOG) from waste water treatment plant (WWTP). The goal is a purity of the oil where the Novozymes Eversa enzymatic process can produce biodiesel from the convertible material. Any industrial technology for producing biodiesel from FOG have until now been focused of using acid esterification and caustic transesterification, in an array of different setups. The main issues being high free fatty acid and

catalyst side reactions. After the ban on high sulfur (500ppm) fuel, things have slown down. The FOG harvester is not just a piece of equipment, but a mobile lab and design team, who will verify and prove in pilot scale the functionality of a specific self-sustaining piece of equipment for a location. The main challenge of the FOG harvester has been to identify the enzymatic process' key parameters. Just removing water and solids is not enough; pH-control and demulsification are also key, resulting in a feedstock for enzymes and not just clean oil. Any carry over of suspended water have the risk of no conversion and a loss of all catalyst. Along with the development of the FOG harvester, Tactical Fabrication has been a key player in the development and improvement of a downstream design, which solves the challenges arising from the use of enzymatically catalyzed biodiesel production and removes the sulfur from the crude biodiesel. A separate issue, which does not practically impact the FOG harvester, is the calculation of yield, and therefore a major challenge in getting the technology implemented. The oily feedstock from a specific WWTP will have a target level on convertible material, so to optimize reactor space, but the biodiesel production will need to handle waste streams, just as it is the case with other oil raw materials. The enzymatic process with distillation currently offers 80% low sulfur biodiesel relative to the convertible material in the FOG. We expect to gather more real-life production data, which will both allow us to optimize the yield and understand the degree of variation associated with WWTP locations. In this presentation we document how high sulfur FOG have been collected from waste water and converted to ASTM biodiesel by an enzymatic biodiesel process.

Industrial Enzymatic Biodiesel from Low-cost Feedstocks Marcelo Cantele*, *Tranfertech Gestão de Inovações LTDA, Brazil*

Currently, large scale production of biodiesel has been mostly based on homogeneous, alkali catalysis. However, biotechnological production of biodiesel with lipases has received growing consideration in recent years and is undergoing a rapid development. Compared to conventional alkali-catalyzed production, the enzymatic process is considered a "green route" because it is less energy intensive and produces higher-purity product with less downstream operations. In addition, the enzymatic process is very tolerant to high acid and water contents present in waste oils and increases the biodiesel yield by avoiding the typical soap formation due to alkaline transesterification. Edible oils with less than 1 wt% free fatty acids (FFA) have been used as feedstock for industrial biodiesel production despite the relatively high cost of the raw material, which is nowadays one of the most significant factors affecting the economic viability of biodiesel production. In order to make the production of enzymatic biodiesel competitive compared to petroleum-derived diesel, feedstock for long-term supply and at the lowest price possible must be pursuit, such as soapstock, acid oils, deodorized distillates, grease trap fat. The use of unrefined, less expensive, high FFA, lower-grade oils and fats would result in a dramatic reduction of the overall costs of enzyme-catalyzed biodiesel production. Liquid formulations of lipases can provide a highly competitive option for the conversion of oils and fats to biodiesel. In this context, the performance of a commercial, low-cost, soluble free-lipase (Eversa Transform 2.0 from Novozymes) is documented in this talk. Low-cost feedstocks such as yellow grease and tallow used in the enzymatic hydrolysis followed by esterification reactions for fatty acid methyl esters (FAME) production, both in

laboratory scale and pilot plant units (1 ton/batch) will be discussed. Results of biodiesel production from an industrial (~ 100 ton/day) plant is also described and details discussed based on a complete flowsheet of the large-scale industrial processing, from the raw materials processed, reaction system, washing, phase separation and purification to give on-spech biodiesel storage. Finally, local biodiesel market is discussed bringing future challenges and perspectives for the field.

Customized Solutions Through Modular Engineering of Renewable Biodiesel Production Plants Gijs Calliauw*, Wim De Greyt, Dario Altera, and Marc J. Kellens, *Desmet Ballestra Group, Belgium*

While extensive R&D has convincingly demonstrated the potential of enzymatic biodiesel production to become a credible and economically attractive process, the next challenge is to extend and improve the engineering and safe operation of such plants at an industrial scale. Compared to chemical catalysts, enzymes allow the use of a wider spectrum of feedstocks, containing higher amounts of free fatty acids, water and oxidation products. This advantage can extend the applicability and profitability of the total process, but it also requires that the actual plant can effectively deal with this large variety. Also, an industrial biodiesel plant involves more processing steps than only the methylation section, and if any enzymatically based technology is to be commercially viable, it requires the flexibility to be integrated into existing biodiesel plants as well. Hence, a successful industrial design of the enzymatic biodiesel process is one that can handle the feedstock variability as well as be able to blend in seamlessly with current practices in existing installations. Desmet Ballestra now approaches this challenge through a 'modular design' in which the sequence of processing the oil feedstock to in spec biodiesel, as well as the side stream handling, is broken down in many processing steps. This facilitates engineering and design of customized installation and the understanding of the sensitivity of each processing step. Such approach is also of crucial importance in assessments of the return-on-investment, the impact of feedstock changes on yields, and the importance of waste stream valorization. Modular engineering thus helps to understand the sensitivities and impact of various process choices on the final feasibility of an enzymatic biodiesel project, how it compares to existing competing chemical technologies, and therefore contributes to building better plants in response to the customer's specific demands.

Enzymatic Biodiesel from Distiller's Corn Oil. Experiences from Full Scale Production Anders Rancke-Madsen*[1], Mark Bollinger[2], Hans Christian Holm[3], and Per Munk Nielsen[1], *[1]Novozymes, Denmark; [2]Novozymes, USA; [3]Novozymes A/S, Denmark*

The use of liquid enzymes in biodiesel production has been a break-through as liquid enzymes can handle crude feed-stocks with any content of free fatty acids at low economical risks. However, complexity of re-using the enzyme and lack of robust polishing technologies has been major challenges. A next version liquid enzymatic biodiesel process is based on a new more stable variant of the Thermomyces lanuginosus lipase, single time use of the enzyme and a polishing neutralization step called "the one reactor process". The new process has over the last two years been validated in large scale and has proven robust due to simplicity, low process variations and excellent separation performance. Reaction conditions on distiller's corn oil are 3 kg enzyme/ton oil, 2% water, 1.2-1.5 equivalents of methanol and 24-30 hours of reaction time at 104°F/40°C. After reaction, dilute caustic is mixed

in to the reaction mixture for 30 minutes. After a few hours of settling time at 130°F/60°C, the FAME phase is separated from the heavy phase, washed with 2% water, dried and distilled. The biodiesel yield is 93% and the glycerol heavy phase has relatively low salt and methanol content. The presentation will discuss the process parameters in detail and document the overall process robustness with data from lab as well as full scale production.

Eurofins QTA, AOCS Ck2-09 Solution for the Quality/Process Control in Enzymatic Biodiesel Production Nan Wang[1], and Kangming Ma*[2], *[1]Eurofins Analytical, USA; [2]Eurofins QTA Inc., USA*

Process control is critical to produce high quality biodiesel in the enzymatic process. Traditional analytical methods are based on the wet chemistry or chromatographic methods. These methods require tedious sample preparation and lengthy analytical time. The operators' error can also make the results unreliable. AOCS ck2-09 is based on the patented technology utilizing the FT-MidIR instrumentation. It analyzes multiple parameters without sample preparation. All results are generated within two minutes. The calibration database has covered all the feedstock available in the market and monitored constantly. The rapid results can be used closely to monitor the enzymatic process from the feedstock, transesterification, to the polishing stage, ensuring the quality of the B100. The presentation will demonstrate the operation of QTA system and the best practice for enzymatic biodiesel production.

BIO 4: Plant Lipid Biotechnology and Genomics

Chairs: Richard Wilson, Oilseeds & Bioscience Consulting, USA; and Thomas A. McKeon, USDA, ARS, WRRC, USA

Introduction of Reduced Total Saturated Fats High Oleic Canola Hybrids in North America Xinmin Deng*, *Cargill Inc., USA*

Objective: Breeding reduced total saturated fats high oleic canola hybrids Methods Used: Hybrid canola breeding, genomics and molecular markers assistant breeding Results: Cargill invests in a vertically integrated high oleic canola seeds and oil supply chain globally, started introduction of high oleic canola seeds and oils in the early 1990 in North America, converted Major Food Service Industry frying oil with trans-free high oleic CV65 oils in 2007, and introduced CV65 oil in Australia and New Zealand in 2010. Cargill invests its global high oleic canola breeding program to provide competitive yield and enhance sustainability to supply high oleic oils globally to its customers over 20 years. In 2017 Cargill will introduce a brand new high oleic canola hybrid in North America with reduced total saturated fats (C12:0+C14:0+C16:0+C18:0+C20:0+C22:0+C24:0)les s than 4.5% vs regular canola or high oleic canola at 7.0% without sacrifice any frying stability and functionally. Cargill has invested in modern genomics and molecular markers technology, in combination with its leading yield canola hybrid system; it has demonstrated consistent yield and stable fatty acid profile of its new reduced sats high oleic canola across its Western Canada commercial production regions. Conclusions: After 2020, with its significant investing in its global breeding program, Cargill will be able to supply reduced saturated fats high oleic canola seeds from its global production bases and leverage its global oil supply chain to its customers in China and Asian Pacific regions and European countries.

Investigation of Exotic Fatty Acid Biosynthesis: Transcript Profiling and Biochemical Characterization of Lipid Metabolic Genes from *Litchi chinensis* Seeds Jay Shockey*[1], David Kuhn[2], Tao Chen[3], Catherine Mason[4], and Barbara Freeman[2], *[1]SRRC-ARS-USDA, USA; [2]USDA-ARS Subtropical Horticultural Research Station, United States; [3]Shenzhen Fairy Lake Botanical Garden, The Chinese Academy of Sciences, China; [4]SCCR-ARS-USDA, USA*

Traditional agronomic seed oils contain five to six common fatty acids that have been optimized primarily for human and animal nutrition. However, nature contains hundreds of examples of novel fatty acids that contain unusual and often highly reactive side-chain functional groups. A few species of oil-accumulating plants produce oils rich in fatty acids containing carbocyclic rings. The energy of the constrained ring structure easily lends itself to industrial production of branched chain fatty acids. Both carbocyclic and branched fatty acids are valuable feedstocks in the production of numerous industrial products. Like many other exotic oilseeds, most plants that produce these types of unusual fatty acids are not amenable to industrial-scale agronomic cultivation and processing. Attempts to engineer carbocyclic fatty acids in transgenic plants are few, and have met with limited success, in part due to very little knowledge of the underlying biochemical mechanisms and potential bottlenecks to the process. In this report, we present data describing the transcript profiles of lipid biosynthetic genes from *Litchi chinensis*, a tropical plant that produces oil containing up to 40% carbocyclic fatty acids. Transcript levels from multiple stages of seed development were compared to that in leaves and flowers to search for isozymes that might be specifically involved in seed oil metabolism.

Enzymes catalyzing the terminal step in triacylglycerol production were also characterized biochemically by expression in transgenic yeast and model plants. The preliminary findings of these studies are described here.

Plant Acyl-CoA-Binding Proteins Function in Stress Protection of Transgenic Plants Mee Len Chye*, *School of Biological Sciences, University of Hong Kong, China*

Acyl-CoA-binding proteins (ACBPs) display conservation at the acyl-CoA-binding domain which facilitates binding to acyl-CoA esters. The genes encoding ACBPs in the model plants, *Arabidopsis thaliana* (thale cress) and *Oryza sativa* (rice), belong to gene families and six members have been reported in each of these species. It has been observed that several genes encoding ACBPs in both *A. thaliana* and *O. sativa* are induced by various forms of abiotic and biotic stresses, including drought, heavy metals, low temperature and phytopathogens. When some of the Arabidopsis ACBPs were overexpressed in transgenic plants, they were conferred stress protection. Studies using acbp knock-out mutants also supported the role of ACBPs in stress responses. Furthermore, a few of these ACBPs contain ankyrin repeats and kelch motifs, which enable interactions with protein partners. Interestingly, the protein partners of the ankyrin-containing AtACBP2 were demonstrated to be stress-responsive proteins, and were subsequently shown to enhance stress tolerance in transgenic plants. The role of AtACBPs in binding ligands and in stress protection will be discussed. Funded by the Research Grants Council of Hong Kong [HKU765813M & HKU17105615M]

Synthetic Biology to Engineer Novel Oils with Enhanced Properties Timothy P. Durrett*, *Kansas State University, USA*

Acetyl-TAGs are unusual triacylglycerols (TAG) with an *sn*-3 acetate group and therefore possess different physical properties compared to conventional TAG. For example, acetyl-TAGs possess a lower viscosity and improved cold temperature properties relative to other vegetable oils, suggesting the use of these molecules as improved biofuels or biodegradable lubricants. In addition, acetyl-TAGs are similar in structure to the emulsifier ACETEM used in foods, and to plasticizers used for PVC food packaging. EaDAcT, a diacylglycerol acyltransferase (DGAT) with *sn*-3 acetyltransferase activity, synthesizes the acetyl-TAGs in the seeds of Euonymus alatus (Burning Bush). Expression of EaDAcT in *Camelina sativa*, combined with the RNAi-mediated suppression of endogenous TAG biosynthesis, led to acetyl-TAG levels as high as 85 mol% in the best transgenic lines. These high acetyl-TAG levels were stable across multiple generations and did not affect seed viability. Through the identification and expression of high activity DAG acetyltransferases from other species, and by modifying the supply of substrates for EaDAcT, we have been able to increase the levels of acetyl-TAGs in transgenic Camelina. To expand the functionality of acetyl-TAGs, we have combined their production with the synthesis of medium chain fatty acids (MCFA). Such acetyl-TAGs with MCFA at the *sn*-1/2 positions are predicted to possess further reductions in viscosity. Electrospray ionization mass-spectrometry (ESI-MS) revealed that MCFA were incorporated in acetyl-TAGs, albeit at a low efficiency. The low levels of acetyl-TAG containing MCFA reflects the poor incorporation of MCFA into the *sn*-2 position of the molecules, as well as the substrate preferences of EaDAcT.

The Genome Sequences of the Ancestors of Cultivated Peanut David J. Bertioli*, *University of Georgia, USA; University of Brasilia, Brazil*

Cultivated peanut (*Arachis hypogaea* L.) is an oilseed and grain legume that is widely cultivated and particularly important as an energy and protein source for smallholders in Africa and Asia. It is an allotetraploid with closely related component genomes that diverged only about 3 million years ago. Together with its large size, ~2.7

Gbp, this makes the assembly of the A. hypogaea genome very challenging. Here we report the use the genomes of the two most probable diploid ancestors of peanut (A. duranensis and A. ipaënsis, sequences produced by the Peanut Genome Consortium) as a "prototype" or "scaffold" onto which sequence reads of cultivated peanut can be overlaid. We show that the genome sequences of the diploid species are very similar to the component genomes of cultivated peanut and use them to elucidate the impact of a degree of segmental allotetraploid genetics in the cultivated peanut genome. Remarkably, considering the extremely high DNA sequence identity of A. ipaensis and cultivated peanut, the species' peculiar habit of depositing their seeds under the ground, and biogeography, we conclude that present day A. ipaënsis is likely a remnant of the very same proto-domesticated population that contributed one of the component genomes to cultivated peanut about 10,000 years ago.

Re-Introducing the Castor Plant for Domestic Production of Castor Oil Tom McKeon*, *USDA, ARS, WRRC, USA*

Castor oil is the only commodity seed oil containing the hydroxyl fatty acid ricinoleic acid. The oil serves as a chemical feedstock for a range of products including lithium grease, polyamide 10,10, polyamide 11 (RilsanTM), surfactants, and a numerous other products. However, the seed also contains the protein toxin ricin and the presence of this toxin has served as a deterrent to domestic cultivation and processing. We and others have developed several approaches that reduce or eliminate any risk from ricin. Our research includes application of breeding to reduce ricin content, physical inactivation of ricin prior to processing and enzymatic digestion during processing. There are very good possibilities for growing castor in the US, but re-introduction will require careful oversight of the crop through processing.

Biosynthetic Mechanisms of Very Long Chain Polyunsaturated Fatty Acids in Microorganisms Dauenpen Meesapyodsuk, Xi Xie, and Xiao Qiu Department of Food & Bioproduct Sciences, University of Saskatchewan, Canada

The de novo biosynthesis of nutritionally important omega-3 very long chain polyunsaturated fatty acids (VLC-PUFAs) such as docosahexaenoic acid (DHA, 22:6-n3) and eicosapentaenoic acid (EPA, 20:5-n3) occurs mostly in microorganisms through either an aerobic pathway using desaturases and elongases or an anaerobic pathway using a polyketide synthase-like PUFA synthase. Thraustochytrim, a unicellular protist, is known to produce a high level of DHA in both membrane and storage lipids. The genes in the aerobic pathway encoding Δ4, Δ5, Δ6 and Δ12 desaturases and elongases were cloned from the species and functionally analyzed in yeast and/or plants. In addition, the genes encoding three subunits of a PUFA synthase and a phosphopantetheinyl transferase (PPTase) for attaching a phosphopantetheine prosthetic group to acyl carrier protein (ACP) domains of the PUFA synthase were also cloned from Thraustochytrium and functionally expressed in Escherichia coli. This presentation will discuss the relative importance of the two pathways in the biosynthesis of VLCPUFAs and highlight our recent progress on functional analysis of catalytic domains of the PUFA synthase.

Metabolically Engineered Plant Oils Surinder P. Singh*, *CSIRO, Australia*

Plant oils are an important source of dietary fat and represent as much as 25% of human caloric intake in developed countries. Plant oils are also extensively used in industry. My talk will discuss strategies being employing in the Plant Oils Engineering Group at CSIRO for the development of nutritionally and industrially improved plant oils. These include the use of RNAi gene silencing for the creation of highly monounsaturated form of Safflower oil for oleochemical applications. I will also discuss the production of long chain omega-3

polyunsaturated fatty acids EPA and DHA, essential for human health, in plant oils. My talk will describe the transition of DHA production in seed of our model species Arabidopsis through to Camelina and our target crop canola. DHA levels that exceed the amount typically found in bulk fish oil have now been achieved in all three species and involved transfer of a 7 gene algal pathway into oilseed crops. Finally, I will describe our efforts to engineer seed oil like levels in leaves of plants. We have used a 'Push, Pull, Protect and Package' strategy to engineer 30% plus oil /drywt in tobacco leaves. This approach has the potential to revolutionise the way plant oils are produced with oil palm like productivity achievable in high biomass C4 crops like sorghum and sugarcane. We believe production of oil in leaves and stems and other non-seed tissues of crops will go a long way to meet the increasing demand for plant oils in the coming decades.

BIO 4.1 / S&D 4.1: Biosurfactants, Bio-derived Surfactants, and Biodetergents

Chairs: Heather Byrne, Huntsman Performance Products, USA; Douglas G. Hayes, University of Tennessee, USA; and Daniel Solaiman, USDA, ARS, ERRC, USA

Tailoring of Mannosylerythritol Lipids by Pseudozyma Species Using Different Renewable Feedstocks Susanne Zibek*, *Fraunhofer IGB Institute for Interfacial Engineering and Biotechnology , Germany*

Currently, sustainable surfactant products with decreased carbon footprint and complete biodegradability are highly demanded. This requires the introduction of new compounds and production processes based on renewable resources. Microbial biosurfactants meets organic criteria and shows also a broad spectrum of molecule diversity. Mannosylerythritol lipids (MEL), produced by fungi of the genus Pseudozyma and Ustilago, are among the most promising microbial biosurfactants with application potential in personal care, technical uses and pharmaceuticals. MEL can be composed of a group of four variants (A to D), which are classified by the degree of acetylation, which affects the polarity of the biosurfactant and by that, their spectrum of application. Depending on the strain and feedstock (fatty acid chain length, unsaturation), unique MEL mixtures can be produced. Therefore, we evaluated the effect of different feedstocks on the production of MEL by various Pseudozyma strains. The results are used to develop strategies for structure-tailoring of the surfactants by enzymatic, process engineering or metabolic engineering methods. This way a portfolio of MEL-derivatives with enhanced hydrophilicity or hydrophobicity was generated. The created portfolio of MEL-derivatives ranges from diacylated and acetylated molecules with hydrophobic properties especially suitable as emulsifier and cosmetic ingredients to monoacylated hydrophilic MEL for applications in aqueous solution. In order to make more types of biosurfactants in sufficient amounts and desired performance available, we are developing scalable production processes (fermentation and downstream processing) for several glycolipids. Our current optimized fermentation processes deliver product concentrations more than 120 g/L for mannosylerythritol lipids.

Integrated Bioprocess Design for the Production of Tailor-made Glycolipids Using *Starmerella bombicola*: Promising Results from Application Testing Lisa Van Renterghem[1*], S. Roelants[1,2], N. Baccile[3], K. De Schamphelaere[4], M. Höfte[5], Q. Christiaens[1], M. Hartmann[1], S. Verweire[1], and W. Soetaert[1,2],*[1]Ghent University, Centre for Industrial Biotechnology and Biocatalysis, Ghent, Belgium, [2]Bio Base Europe Pilot Plant, Ghent, Belgium, [3]Université Pierre et Marie Curie, Laboratoire de Chimie de la Matière Condensée, Paris, France, [4]Ghent University, Environmental Toxicology Unit, Ghent, Belgium, [5]Ghent University, Phytopathology Unit, Ghent, Belgium*

Biosurfactants are an emerging class of surfactants produced by microorganisms, offering a more environmentally friendly alternative compared to traditional surfactants. One type of glycolipid biosurfactants are sophorolipids (SLs), naturally produced by the non-pathogenic yeasts from the Starmerella clade in high amounts (> 200 g/L), explaining its large industrial interest. Due to unique expertise gathered at InBio.be, Starmerella bombicola can be genetically engineered to alter the production towards one specific sophorolipid or novel glycolipid, transforming S. bombicola into a real platform organism.

This research focuses on developing an integrated bioprocess design (IBPD) strategy for

the production of new-to-nature glycolipids using genetically engineered S. bombicola strains. In this strategy, the entire innovation chain is considered: from genetic engineering to medium optimization, fermentation and downstream processing, to final application testing.

The application testing is very important to define possible applications of the tailor-made molecules. Since biosurfactants can be employed in so many fields of industry, this is a complicated task, and therefore a multidisciplinary collaboration was set up. Different possible applications of tailor-made glycolipids were assessed and some very interesting leads were found, showing that there are real opportunities in various markets/applications. For example, a new method to encapsulate iron oxide nanoparticles into liposomes was discovered. Antimicrobial characteristics were assessed for various tailor-made glycolipid molecules for selected bacteria and fungi. An ecotoxicological evaluation of the novel-made glycolipids display much higher (or even not-determinable) EC50 concentrations compared to traditional surfactants, making them very promising alternatives.

This portfolio of tailor-made sophorolipid biosurfactants with varying characteristics and properties will lead to an improved market penetration of biosurfactants in the future.

Microbial Biosurfactants, from Lab to Market: Hurdles and How to Take Them Sophie L.K.W. Roelants*[1], Bernd Everaert[1], Emile Redant[1], Brecht Vanlerberghe[1], and Wim Soetaert[2], *[1]Bio Base Europe Pilot Plant, Belgium; [2]Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Ghent University, Belgium*

Microbial biosurfactants have been holding the promise as thé environmental friendly alternative for petrochemical derived surfactants for many years. The real lift off of this technology is still expected, but some important recent developments were done. On one hand, large companies are investing in this technology and a few products can actually be found on the market today. Dedicated and valorization oriented research at Universities on the other hand has enabled the generation of potent microbial strains, ready to move ahead in the innovation chain. In this paper, we will use an example to show how the integration of process (fermentation and purification) development, optimization, scale up and application testing has been key for biosurfactant technologies to move further ahead in the innovation chain. The production of a new type of sophorolipid, by a strain with lower inherent productivities compared to the wild type sophorolipid producing organism, was optimized by process development and scale up. This resulted in a substantial (x4) increase of the productivity and thus a significant reduction of the production price. Moreover, scaling up the process enabled us to generate large samples for dedicated application testing and perform both a techno economic analysis (TEA) as a life cycle assessment (LCA), sometimes resulting in surprising findings. Sensitivity analysis of the TEA and LCA studies enabled us to identify hotspots for price and impact reduction respectively. The combination of the described efforts and strain engineering is expected to result in a real commercial breakthrough of microbial biosurfactant the coming years.

Sophorolipids in Hard Surface Cleaning Applications Zheng Xue*, Dennis Parrish, Jeff Davidson, Samuel Christry, Andras Nagy, Miyako Hisamoto, and Terrence Everson, *Evonik Corporation, USA*

Microbial biosurfactants produced by fermentation exhibit favorable properties such as low toxicity, skin mildness, and biodegradability. In particular, there is significant commercial interest in sophorolipids, owing to the nonpathogenic

character of the production host and the high yields. Sophorolipids are glycolipids biosurfactants consisting of a sophorose sugar head and a hydrophobic fatty acid tail. The carboxylic end of this fatty acid can be free, forming the acidic structure, or internally esterified at the 4 position of the sophorose head, forming the lactone structure. The lactone form is only stable at neutral or slightly acidic conditions due to the hydrolysis of ester bond at high alkalinity. The narrow pH range for stability against hydrolysis poses significant challenges for utilizing sophorolipids in hard surface cleaning formulations, which are usually formulated at alkaline conditions of pH ≥ 10 to saponify fatty deposits. Sophorolipids formulation at neutral pH with cleaning performance comparable to conventional high pH cleaners were developed, through tuning the hydrophilic-lipophilic balance of the formulation to obtain strong emulsification. The effects of sophorolipids on detergency are investigated using interfacial rheology and interfacial tension measurements. Formulation procedures and comparative results will be discussed.

Sophorolipid Biosurfactant Against Bacteria Relevant to Tooth Caries and Skin Hygiene Daniel K.Y.Solaiman*[1], Richard D. Ashby[1], Joseph Uknalis[2], Aixing Fan[3], and Laurence Du-Thumm[3], *[1]USDA, ARS, ERRC, USA; [2]USDA, ARS, ERRCA, USA; [3]Colgate Palmolive Co., USA*

Sophorolipid (SL) is glycolipid biosurfactant produced by yeast. Its general antimicrobial activity was previously reported. In this paper, we present the antimicrobial activity of SL specifically against oral and skin bacteria. Using a microplate to continuously monitor cell growth, we found complete inhibition of cell growth at SL concentrations ≥1 mg/ml (1,000 ppm) for oral *Lactobacilli* tested and ≥50 µg/ml (50 ppm) for the oral *Streptococci*. SEM study of SL-treated *L. acidophilus* (overnight; 1 mg/ml) suffered extensive cell lysis; *S. mutans* (at SL=130 µg/ml) showed extensive lesions on cell surface but no lysis. SL (at *Lactococci*, as shown by increased cell-doubling time (Td) and decreased final cell density (by A^{600nm}) in concentration-dependent manner. SL at *Streptococci*, as evidenced by a prolonged lag-time of growth curves in a concentration-dependent manner but no differences in T^d and the final A^{600nm}. Standard Minimal Inhibitory Concentration (MIC) test was also performed towards a broad array of oral and skin bacteria. Superior antibacterial properties were achieved against 3 oral *Streptococci* species tested (MIC < 4ppm). Good antibacterial properties (MIC=19-39 ppm) were also achieved towards some Gram-positive skin bacteria such as *S. haemolyticus*, *C. striatus* and pathogenic *S. aureus*. However, its efficacy towards Gram-negative *E. coli* is only moderate (MIC=312-625 ppm). In conclusion, the results presented demonstrated the high value of SL as antimicrobials for applications in oral and skin care industries.

A Journey to Standardisation of Bio-based Surfactants in Europe Juergen G. Tropsch*[1], Christophe Sené[2], Thierry Beaudouin[2], Stephen Mudge[3], and Horacio Hormazabal[4], *[1]BASF SE, Germany; [2]Stepan, France; [3]BSI, UK; [4]AFNOR, France*

The European Commission has decided in 2011 to become the first bio-based economy. In the following, the EU issued a mandate to CEN to develop a standard on bio-based surfactants among other product groups. A new working group within CEN was created to deal with the standardization process (CEN/TC-276 WG3). The working group issued the technical specification CEN/TS 17035 which will be published in April 2017. The CEN/TS 17035 specifies the thresholds on the biomass content (5%, 50% and 95%) and the

naming as well as the methods to determine the thresholds (e.g. radiocarbon method according to EN 16640). The reasoning for the thresholds als well as our approach on the environmental and societal criteria will also be explained. Further work of the working group will include the finalization of a European Norm (EN) and a Technical Report (TR) in 2017. There is planed also an ISO standard on bio-based surfactants. The standard might be used in European ecolabels and in public procurement.

Oil Seed-extracted Oleosome Emulsifiers for Sun Protection Products Soo In Yang*[1], Shuanghui Liu[1], Geoffrey Brooks[1], Yves Lanctot[1], and James V. Gruber[2], *[1]Botaneco Inc., Canada; [2]Botaneco Inc., USA*

As a repository of new life energy source in oil seeds, oil bodies or oleosomes are structurally unique due to their uniformly embedded protein stabilizers into the phospholipid-surrounded triacylglycerol core. This naturally-engineered structure provides a foundation for the biochemically programed collapse and release of oil from the oleosomes in a tightly controlled manner. The oil in the oleosomes are dispersed in the aqueous phase of the cells with the help of the physically stabilized structure by the surface proteins. This structural benefit yields a protective mechanism against coalescence of oil droplets; thus, also increasing the surface area. These physico-chemical barriers provide steric and electrostatic stabilization to the oleosomes, leading to a stable emulsion system. Oleosome itself is an oil-in-water emulsion, but it also possesses an excellent surface active characteristic, thus suggesting its great potential as an emulsifier. We extracted oleosomes using a patented non-solvent-based aqueous extraction process. The purified mixture of oleosome-containing liquid fraction in water with D-glucono-1,5-Lactone, sodium benzoate, and citric acid, has been studied for its potential use as an emulsifier for sun protection products. Our clinical studies demonstrated oleosomes are hypoallergenic and emollient with high water resistance. When applied for sun protection products, where active ingredients are oil soluble UV filters, oleosomes resulted in great emulsion stability and outstanding functionality, specifically boosting sun protection factor (SPF) significantly. This outcome was delivered by diminishing the use of the aggressive UV filters by up to 9 - 10 folds, achieving the same levels of SPF as market-leading products.

The Antibacterial Property of Fatty Acyl Glutamic Acid and Proposed Mechanism Buddhi Lamsal, and Kangzi Ren*, *Iowa State University, USA*

Fatty acyl Glutamic acid (FA-Glu), a highly water-soluble acyl lipoprotein biosurfactant produced by bacterial fermentation, was studied as an antibacterial agent against foodborne-pathogens. The objective of this study was to determine the how the FA-Glu interacted with bacterial cell membrane to achieve bactericidal effect. The minimum inhibitory concentrations of FA-Glu and other bio-based surfactants against E.coli O157:H7, Salmonella enteria and Listeria monocytogenes were determined and compared. The mechanism of FA-Glu antibacterial property was studied. Cell content leakage test by spectrometry indicated FA-Glu caused significant leakage of cytoplasmic protein and DNA. The differential scanning calorimetry study of FA-Glu interaction with artificial cell membrane revealed that FA-Glu disrupted the cooperativity of phospholipid bilayer structure by interacting with the hydrocarbon chain, reducing phase transition temperature and enthalpy change. The interaction with different types of phospholipids indicated that bio-based surfactant were more effective against Gram-positive bacteria compared to Gram-negative ones. The major phospholipid (DPPE) in

Gram-negative E.coli O157:H7 is harder to disturb than the major phospholipid (DMPG) in Gram-positive Listeria monocytogenes. Composition of various proteins (from cytoplasma, cell membrane and cell wall) of FA-Glu treated and control bacteria will be examined by SDS-PAGE to determine whether FA-Glu interacted with a specific protein or show the detergent solubilization effect. The study will provide information on possible development of disinfectant formulation using this novel bio-based surfactant.

Triglyceride Derived Surfactants and Interesterification: Synthesis and Performance Properties Heather E. Byrne*[1], George A. Smith[2], and Angela Garibay-Lewis[2], *[1]Huntsman Performance Products, USA; [2]Huntsman Corporation, USA*

Castor oil ethoxylates (COEs) have been widely used for emulsification properties in industries such as agriculture, metal working and personal care. The main technology available on the market uses direct ethoxylation on castor oil to obtain this vegetable based surfactant. Although the older technology is still used today, in the more recent years, it was found that you could obtain castor oil ethoxylates by scrambling ethoxylated glycerin and castor oil triglycerides. Compared to the old technology, this new route helps to keep both the hydroxyl group intact and the levels of 1,4-dioxane low. Investigations showed benefits to these newer castor oil ethoxylates which in turn led to further experimentation in order to see if we could derive an estolide with this generation of COE. Once able to synthesize the interester of the castor oil ethoxylate, several performance tests were run to see what benefits, if any, were seen. This data along with other comparison data will be discussed in depth.

BIO 5: General Biotechnology

Chairs: Long Zou, Bunge Oils, USA; and Lu-Kwang Ju, University of Akron, USA

Incorporation of Rosemary Extract into Alkylglycerol-based Delivery Systems to Obtain Formulations Highly Bioaccessible and Bioactive Marta C. Corzo-Martinez*[1], Luis C. Vazquez[1], Guillermo Reglero[1], Ana Ramirez[2], and Carlos Torres[1], *[1]University Autonoma of Madrid, Spain; [2]Imdea Food Institute, Spain*

Over the last few years, new formulation strategies have been specifically developed for the oral administration of poor water-soluble active compounds such as natural extracts with high phenolic content. Concretely, in recent years, a great deal of interest has been focused on the incorporation of these products into lipid-based delivery systems (LBDS), which has shown to be one of the most powerful strategy to increase their clinical efficacy. In this sense and attending to the multiple biological activities of alkylglycerols (AKG), their utilization as LBDS provides additional interest. The aim of this work was to study the capability of AKG to act as efficient LBDS of a rosemary extract to enhance their bioactivity. For that matter, an AKG-based delivery system (ABDS) was combined with the mentioned extract and bioaccessibility and bioactivity of the formulations were assessed. An in vitro intestinal digestion model simulating in vivo conditions was used for digestibility and bioaccessibility studies and antiproliferative activity was determined using human pancreatic and colon cancer cells. Formulations of ABDS with rosemary extract showed improved bioaccessibility and higher antiproliferative effect than the non-formulated extract alone. The use of AKG as LBDS is, therefore, an efficient approach to increase the bioefficacy of natural extracts, leading to formulations highly bioaccessible and bioactive.

Enrichment of γ-Linolenic Acid from Evening Primrose Oil Using a Self-immobilized *Candida rugosa* Lipase Glory Chidi Chijioke*[1], Heejin Kim[2], and In-Hwan Kim[3], *[1]Korea University, South Korea; [2]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea; [3]Korea University, Republic of Korea*

γ-Linolenic acid (GLA, all-*cis*-6,9,12-octadecatrienoic acid, C18:3, n-6) is a vital n-6 fatty acid which modulates immune and inflammatory responses. In this study, GLA was enriched successfully via an immobilized lipase-catalyzed esterification of alcohol and fatty acid from evening primrose oil in this study. Four alcohols were screened for esterification and the most suitable alcohol was lauryl alcohol. The immobilized lipase was prepared from a free type *Candida rugosa* lipase. Seven carriers were tested and Accurel MP 1000, the most suitable carrier was selected for immobilization. The effects temperature, enzyme loading, and initial water content were investigated in a batch reactor. Optimum conditions were a temperature of 15°C, an enzyme loading of 4% (based on the total substrate weight), and an initial water content of 0%. Under these optimum conditions, GLA was enriched from 9.1% of initial sample to 54.7%. A packed bed reactor was also employed to enrich GLA and was compared to the batch reactor.

Preparation of High Purity 2-Monopalmitin from Lard by Solvent Fractionation and Enzymatic Ethanolysis Son Woo Kim*[1], Hye Ryung Park[2], Nakyung Choi[2], and In-Hwan Kim[3], *[1]Dept. of Public Health Sciences, Graduate School, Korea University, South Korea; [2]Korea University, South Korea; [3]Korea University, Republic of Korea*

2-Monopalmitin (2-MP) is a desirable substrate

for the synthesis of human milk fat substitute. High purity 2-MP was successfully prepared from lard by solvent fractionation and enzymatic ethanolysis in this study. Lard solid fraction (LSF), which had a high content of palmitic acid at *sn*-2 position, was isolated by solvent fractionation using acetone. The content of palmitic acid at the *sn*-2 position of LSF increased from 69% of initial lard to 84% after solvent fractionation. 2-Monoglyceride was produced from LSF via Novozym 435-catalyzed ethanolysis and the optimum reaction conditions were a temperature of 30°C, a solvent ratio of 1:5 (LSF to ethanol, w/w), and an enzyme loading of 5%, respectively. Consequently, high purity 2-MP (ca. 93%) was obtained from the reaction mixture by solvent fractionation using *n*-hexane.

Enzymatic Modification of Menhaden Oil to Incorporate Capric Acid Sarah A. Willett*, and Casimir C. Akoh, *University of Georgia, USA*

The objective of this study was to produce structured lipids (SL) with menhaden oil and capric acid (C10:0) or ethyl caprate. Capric acid or ethyl caprate was blended with menhaden oil and enzymatic reactions were optimized using the Taguchi L9 orthogonal array with three levels of capric acid or ethyl caprate to menhaden oil ratio (0.1:1, 0.3:1, and 0.5:1 [w/w]), three levels of enzyme load (5, 10, and 15% [w/w]), three levels of temperature (40, 50, and 60˚C), and three levels of reaction time (12, 24, 36 h). Non-specific Candida antarctica lipase B (CAL-B), Lipozyme 435 and sn-1,3 specific Rhizomucor miehei lipase, Lipozyme RM IM as biocatalysts were compared in both the acidolysis and interesterification reactions. Total and sn-2 fatty acid compositions, triacylglycerol (TAG) molecular species, thermal behavior, and oxidative stability were determined. Reactions with ethyl caprate incorporated significantly more C10:0 than reactions with capric acid at 28.54±0.42 versus 15.55±1.32 mol%, respectively. Analysis of signal-to-noise ratios in the orthogonal array found the optimal reaction conditions to be substrate ratio of 0.5:1 [w/w], enzyme load of 10% [w/w], 60˚C, and 24 h reaction time for reactions using capric acid as a substrate and reactions with ethyl caprate as a substrate with Lipozyme RM IM as biocatalyst. Optimal reactions conditions using ethyl caprate as substrate with Lipozyme 435 as biocatalyst were substrate ratio of 0.5:1 [w/w], enzyme load of 5% [w/w], 40˚C, and 36 h reaction time.

Soybean Flour Lipoxygenase: Activity on Both Free and Esterified Fatty Acids for Bioactive Compound Synthesis Hoang-Anh T. Tu*, Eleanor P. Dobson, Colin J. Barrow, and Jacqui L. Adcock, *Deakin University, Australia*

Lipoxygenase (LOX) catalyses the controlled oxidation of LC-PUFAs to help form biologically important anti-inflammatory and/or pro-resolving lipid mediators and analogues. Soybean flour (SBF) contains different LOX isozymes and is a stable source of LOX-2, which is unstable when purified. LOX-2 is versatile because it can react with both free and esterified FAs, but is commercially unavailable. In this work, we aimed to use SBF without purification or complex preparation as a LOX source (sfLOX) to synthesise bioactive molecules directly from DHA and trilinolein (TL) (without prior hydrolysis). The sfLOX-DHA and sfLOX-TL reactions were optimised and evaluated using NP- and RP-HPLC, to maximise the yield of compounds containing 1 to 3 hydro(pero)xy groups. Reaction products were characterised by GC-MS, chiral HPLC, UV-visible and NMR spectroscopy. Our results showed that sfLOX had the same efficiency as 15-sLOX-1 (a commercially available soybean enzyme) for synthesising di-hydroxy compounds from DHA: 7*S*,17*S*-dihydroxydocosahexa-4*Z*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-enoic acid (RvD5) and 10*S*,17*S*-dihydroxydocosahexa-

4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid (PDX). The antioxidant BHT was found to increase the sfLOX-TL reaction yield significantly (approximately 4.5 fold). The hydroxy groups on the linoleate moieties in TL were identified as mainly 13*S* (~90%) indicating high regio- and stereo-specificity. This work demonstrated that SBF can be used as an economical alternative to commercial 15-sLOX-1 in synthesising bioactive compounds from both free and esterified FAs for nutraceutical and pharmaceutical industries. It is the first time that SBF has been used to produce di-hydroxylated compounds from DHA, and the first time sfLOX-TL reaction products have been characterised.

BIO-P: Biotechnology Poster Session

Chairs: Byung Hee Kim, Sookmyung Women's University, Korea; and Shigenobu Kishino, Kyoto University, Japan

1. **Enzymatic Approaches for Manufacture of EPA-and-DHA-Enriched Triglyceride Fish Oil** Jiazi Chen*[1], Guanghui Li[1], Yinglai Teng[2], Ying Li[3], and Yong Wang[4],[1]*Dept. of Food Science and Engineering, Jinan University, Guangzhou, China;* [2]*Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, Guangzhou, China;* [3]*Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, China;* [4]*Jinan University, China*

Fish oil is rich in triglycerides of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The currently commercialized triglycerides fish oil generally have low amounts of EPA and DHA (ca. 18% and 12%) and therefore it is necessary to enhance their contents. In this research, enzymes are employed to prepare triglyceride fish oil which is high in EPA and DHA (> 45% in total), providing a promising approach for the fish oil industrial. Using the refined deep-sea fish oil as feedstock, lipase Amano AY“Amano”400SD was selected to effectively conduct the partial hydrolysis of fish oil which leads to an enrichment of EPA and EPA. After molecular distillation, EPA and DHA contents in the heavy phase were 25.3% and 21.6% and their recovery rates were 68.3% and 87.2%. EPA and DHA were also collected from the light phase using urea adduction fractionation, converted into ethyl esters and recycled by enzymatic transesterification. In the final product, 95.9% of triglycerides were obtained with EPA and DHA contents of 25.6% and 17.4%.

2. **Comparison of Stereoselective Hydrolysis of Triacylglycerols by Free Lipases from Thermomyces Lanuginosus and Rhizomucor Miehei and the Determination of Final Products by H-NMR and GC-FID** Ali Reza Fardin-Kia*[1], Clark Ridge[1], and Francisco J. Bueso[2],[1]*US Food and Drug Administration, USA;* [2]*Associated Professor, Honduras*

A number of recent publications have linked possible health effects to mono- or diacylglycerols, while other publications have reported on the importance of the acylglycerols as emulsifiers in the food and cosmetic industry. In this context, there is interest in evaluating lipases, which can be used to produce specific mono and diacylglycerols with differing physical and/or chemical characteristics. The objective of this study was to compare the stereo selectivity of a new soluble 1-3 regioselective lipase, from Thermomyces lanuginosus (Callera), with a lipase from Rhizomucor miehei (Palatase), in the hydrolysis of soybean oil. Additionally, a rapid High-Temperature-GC-FID (HT-GC-FID) method was developed to monitor the products of the hydrolysis. Using the optimum concentrations of Callera (1.5%) and Palatase (4.0%) the direct comparison showed no significant difference in the stereo selectivity of both enzymes. In addition, a new optimized H-NMR method was used to quantify and determine the type of diacylglycerols in the final product.

3. **Preparation of Arachidonoyl Ethanolamide by Enzymatic Amidation of Arachidonic Acid Purified from a Microbial Oil** Xiaosan Wang*, Yingying Wang, Qingzhe Jin, and Xingguo Wang,*Jiangnan University, China*

Arachidonoyl ethanolamide (AEA) is a bioactive lipid naturally occurring in animal and plant tissues. In this study, arachidonic acid (ARA) was enriched firstly from a microbial oil and then used for the enzymatic preparation of AEA by amidation with ethanolamine. ARA was purified by urea complexation and acetonitrile extraction after being converted to free fatty acid. The purity of ARA increased from 44.4% to 85.1% after a two-step purification. The purified ARA was subsequently used for AEA synthesis by the enzymatic amidation. The results showed that under the optimum conditions (10% Lipozyme 435 as catalyst, 3 mL hexane as solvent, 70 °C for 2 h), Fatty acid ethanolamide was obtained in a 95.6% yield. Enzymatic synthesis of AEA by the amidation of ethanolamine with ARA from a fungal oil is effective and practical. Preparation of value-added product by utilizing microbial oils is beneficial to achieve economic sustainability for microbial oil industry.

4. **Relationship Investigation Between Lipid Accumulation and Nitrogen Consumption of C.minutissima Using Special Designed Nitrate Sensor** Nihat E. Balkanli, Ibrahim Isildak, Didem Özçimen, Vildan Erci, and Benan Inan*,*Yildiz Technical University, Turkey*

Nitrogen limitation is the most effective method to increase the lipid content in microalgae. There are lots of studies investigated the effect of nitrogen limitation on microalgal growth and its biochemical composition. However, these studies are usually based on the specific initial conditions of nitrogen source, not on the continuous measurement of nitrogen consumption during microalgal growth. Measurement of the nitrogen consumption in the medium is important to understand when the cells are utilizing nitrogen and producing TAGs. There are still unknown patterns in TAG production which is a significant research area for algae researchers. In this study, investigation of Chlorella minutissima growth with the relationship between lipid accumulation and nitrogen consumption. The nitrate amount in growth medium was measured by using special designed composite nitrate sensor. It was found that lipid content of microalgae increased with the decrease of nitrate in the medium. This condition, also known as the nitrogen limitation, has led to the increase in lipid content of microalgae by protecting itself to environmental and biochemical changes. Results which were also examined statistically are in agreement with other studies in the literature.

5. **Statistical Analysis of the Parameters Affecting the Amount of Bioactive Substances of B. Braunii Microalgae** Benan Inan*, and Didem Özçimen,*Yildiz Technical University, Turkey*

Algae are renewable resources and have various natural bioactive compounds. Recent studies showed that these molecules derived from algae have antiviral, anticancer, antifungal, antibacterial, anti-inflammatory and other pharmacological effects. Botryococcus braunii is a single-cell, slow-growing photosynthetic microalgae of green algae, produces large amounts of biomass, lipid, and carbohydrates which have bioactive properties. Apart from hydrocarbons, it also produces bioactive molecules such as exopolysaccharide and carotenoid. In this study, effect of light cycle, temperature and nitrogen limitation on lipid and β-carotene content of B. braunii was investigated according to the Box-Behnken statistical model. According to the obtained equation, it was seen that lipid content of

microalgae decreased with the increase of nitrogen amount and temperature and it was found that the nitrogen depletion is the most effective parameter in comparison with light cycle and temperature. The light cycle was determined to have positive effect on lipid content. In addition to this, the decrease in nitrogen stress leads to an increase in the amount of β-carotene, and also there was an increase in the amount of β-carotene as the temperature increases. The reason for this, increased temperature induces active oxygen radicals and causes carotene production in microalgae. Nitrogen limitation has also been reported to induce carotene formation in microalgal cells. According to the obtained equation, temperature is determined as the most effective parameter for the β-carotene content of B. braunii microalgae. The results obtained in this study were found to be consistent with the literature.

6. **Identification and Functional Analysis of a Mutant Allele of *Gossypium barbadense* Fatty Acid Desaturase-2** Jay Shockey[1], Michael K. Dowd*[1], Brian Mack[1], Matthew Gilbert[1], Brian Scheffler[2], Linda Ballard[2], James Frelichowski[3], and Catherine Mason[4],*[1]SRRC-ARS-USDA, USA; [2]ARS-USDA, USA; [3]SPARC-ARS-USDA, USA; [4]SCCR-ARS-USDA, USA*

Cottonseed oil has lost market share in recent years due, in part, to less than optimal ratios of constituent fatty acids found in either the native or partially hydrogenated oils. Consumer reluctance to consume dietary trans-fats or genetically modified products has created strong demand for naturally-occurring vegetable oils with high monounsaturated to polyunsaturated fatty acid ratios. A few exotic accessions of *Gossypium barbadense* have been identified that contain the dual beneficial traits of normal fiber content and elevated seed oil oleate content (40-45%). The genome of one such accession was sequenced, and a mutant candidate fatty acid desaturase-2 (*FAD2-1D*) gene was identified. In Arabidopsis leaves, the mutant protein produced significantly less linoleic acid compared with a 'repaired' version of the same enzyme. Development of markers associated with this mutant locus will be useful in current efforts to breed this high-oleate trait into agronomic *G. barbadense* (Pima) and *G. hirsutum* (Upland) cotton varieties.

7. **Comparative Lipidomic Analysis of Schizochytrium Limacinum SR31 Cells Using Different Carbon Sources** Ming Chang*, Tao Zhang, Leilei Li, Qingzhe Jin, and Xingguo Wang,*Jiangnan University, China*

Lipidomics is emerging as a critical factor in understanding of cellular metabolism, but little has been done in the study of oleaginous yeasts. Schizochytrium limacinum sp. accumulates large amounts of polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (DHA). Previous studies showed neutral lipid was preferentially converted to phospholipid and accompanied by a degradation of saturated fatty acids during lipid turnover stage, but the cellular connection between triacylglycerol (TAG) and phospholipid (PL) remains unknown. In this study, the effect of different carbon sources on Schizochytrium limacinum SR31 was investigated by comparative lipidomic analysis. Results showed that the phenomenon of lipid turnover was obvious when using glycerol as carbon source, highest dry cell weight (62.99 g/L), lipid content (51.03%) and DHA content (50.48%) were observed. Lipidomics anlysis indicated that phosphatidylglycerol (PG) and phosphatidylinositol (PI) was the biomarker to differentiate various carbon sources. The level of PG was high when glycerol was using as a carbon source. TAG containing saturated fatty acids, such as 14:0-14:0-16:0, 14:0-16:0-16:0 and 16:0-16:0-

16:0 would be preferentially hydrolyzed in lipid turnover stage. TAG containing PUFA, such as 16:0-22:6-22:6, 16:0-22:6-22:5, 22:6-22:6-22:6 and 22:6-22:6-22:5 would be effectively enriched. Compared with other PL fractions, PI and phosphatidylcholine (PC) was abundant with PUFA and increased with time prolonged, which might indicate that PI and PC played a critical role when lipid turnover happens. A possible migration of PUFA to PI or PC might enhance its resistance to environment changes.

8. **Hypolipidemic Activity of Structured Pinolenic Triacylglycerols in Diet-induced Obese Mice** Min-Yu Chung*[1], Hyo-Kyoung Choi[1], Jin-Taek Hwang[1], Hee-Don Choi[1], and Byung Hee Kim[2],*[1]Korea Food Research Institute, South Korea; [2]Sookmyung Women's University, Korea*

This study aimed to examine hypolipidemic activity and the beyond mechanism of pinolenic acid (PLA) in the pinolenic triacylglycerols with even distribution of the PLA on the glycerol backbone in mice with high fat diet (HFD)-induced obesity. Structured pinolenic triacylglycerols containing 13 mol% (SPT13) and 44 mol% PLA (SPT44) were prepared via a nonspecific lipase-catalyzed esterification of glycerol with free fatty acids obtained from the oil of Korean pine nut. A HFD for 15 weeks caused a significant enhancement of serum triglyceride, total cholesterol, and LDL cholesterol levels, which were significantly decreased by 5% dietary supplementation with SPT44. HFD-fed mice exhibited significantly higher liver weight, which was significantly attenuated by SPT44 supplementation. This reduction was likely attributed to decreased hepatic total lipids, including triglyceride and total cholesterol. To further examine beyond mechanism, expression levels of hepatic genes related to lipogenesis and cholesterol metabolism were measured. SPT44 significantly attenuated DGAT1 that was otherwise increased by HFD. SPT44 also increased LDLR expression levels, which was likely attributed to increased SREBP2 without alteration of PCSK9 by SPT44 in the liver of obese mice. The regulation by SPT44 occurred in the fatty liver of mice was due to greater accumulation of SPT44 in the liver.

9. **Pigment Products Derived from Algae** Deniz Ismik*, Muharrem Bogoclu, and Sevil Yucel,*Yildiz Technical University, Turkey*

Microalgae contains many different pigments that absorb the sunlight for photosynthesis. As a photosynthetic organism, microalgae have three major classes of pigments; chlorophyll, phycobiliproteins and carotenoids. Chlorophylls are green pigments and carotenoids are yellow or orange pigments. Carotenoids are lipophilic and phycobilins are hydrophilic. Xanthophylls and astaxanthin are belong to carotenoid group. Animals and also humans are not able to synthesize carotenoids. So carotenoids must be obtain from plant and microalgae. Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. Synthetic colorants are used in various industries such as; food, cosmetic, pharmaceutical. Because of harmful effects of synthetic colorants, microalgal pigments became more popular. The market for pigments is quite wide. Some microalgae spices has been used as commercial pigment source. For example, blue phycocyanin from Spirulinaplatensis is a natural pigment for use in cosmetics and some foods. Biliproteins are also can extract for commercial usage from Spirulina and Porphyridium species. There are various factors effecting pigment production in microalgae spices such as pH, temperature, salinity, light intensity, growth medium. In this review, the pigments studies from microalgae have been presented.

10. **Production of Omega-3 Fatty Acids EPA and DHA from Microalgae** Ali can C. Ozarslan*, Sevil Yucel, and Yeliz Elalmis,*Yildiz Technical University, Turkey*

Omega-3 fatty acids have important biochemical and physiological activities in the body. One of the important sources of the omega-3 fatty acid is microalgae. The major fatty acids in most microalgae cells are palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) or α-linoleic acid (C18:3). Whereas some microalgae species are able to produce long chain polyunsaturated fatty acids such as Eicosapentaenoic acid (EPA) (20:5ω-3), Docosahexaenoic acid (DHA) (22:6ω-3). Each species has its own fatty acids and nutrient content. For example, in autotrophic conditions, 39% of the total fatty acid in Phaeodactylum tricornutum and Nannochloropsis sp. is EPA. Similarly, in heterotrophic conditions, Thraustochytrium and Schizochytrium limacinum contains between 30 and 40% DHA level. EPA and DHA compositions are affected by microalgal life cycle, physicochemical conditions such as temperature, pH, aeration rate, medium composition, stress conditions, salinity, illumination intensity. Significant amount of research has been performed to investigate EPA and DHA production in microalgae. In this study, these researches will be reviewed in detail.

11. **Enzyme-assisted Extraction of Njangsa (*Ricinodendron heudelotti*) Seed Oil** Stephen E. Lumor[1], Samuel A. Besong[1], Alberta Aryee[2], and Immaculate T. Arrey*[2],*[1]Dept. of Human Ecology, College of Agricultural Sciences, Delaware State University, USA; [2]Delaware State University, USA*

Solvent extraction methods are widely used industrially to obtain oil from plant seeds, mostly due to their high efficiencies (90-98% w/w yield). However, consideration is being given to solvent-free extraction methods due to the growing concern that residual solvent in oil could pose significant health risks. As such, enzymatic extraction methods are receiving considerable interest in the oil industry due to their high specificity and low operating cost. Enzymes are known to hydrolyze and degrade the cell wall of oilseeds, which significantly increases oil yield and quality upon extraction. Therefore, the aim of this study was to explore the effects of five different enzymes on the quality and yield of Njangsa(*Ricinodendron heudeulotti*) seed oil. To extract the oil, 50 g of ground Njangsa seeds was treated with each enzyme (2% w/w of hemicellulase, cellulase, protease, pectinase, and amylase). The treatments were incubated for 24 hours at the specified pH and temperature of each enzyme, with constant stirring (120 rpm). The resulting enzyme digest was centrifuged at 4000 RCF for 20 minutes to separate residues (enzymes and seed particles), followed by filtration to obtain the oil. Our initial findings showed that the enzymes had differing effects on oil yield. The highest yield was obtained with hemicellulose, followed by amylase, cellulose, pectinase and protease. Ongoing work is assessing quality indices of oils obtained by enzyme-assisted and solvent extraction methods.

12. **Enzymatic Interesterification of Coconut and High Oleic Sunflower Oils for Edible Film Application** Casimir C. Akoh, and Maria A. Moore*,*University of Georgia, USA*

Three blends (60:40, 70:30, and 80:20) of coconut (CO) and high oleic sunflower oils (HOSO) were interesterified using an immobilized enzyme, Lipozyme TL IM. The three structured lipids (SLs), HOCT6 (60:40), HOCT7 (70:30), and HOCT8 (80:20), were compared to the initial oils to determine the best SL for application in an edible film. Products were compared based on fatty acid profile, TAG molecular species, melting profile, moisture vapor

analysis, mechanical properties, film transparency, and thickness, to determine the best SL for the edible film. Interesterification increased the amount of oleic acid at the sn-2 position compared to the physical blends. CO had 5.50 ± 1.67 mol% oleic acid at the sn-2 position, and when interesterified with HOSO (92.81 ± 1.10 mol% oleic acid) the amount of oleic acid significantly increased ($p<0.05$) at the sn-2 position for each product HOCT6, HOCT7, HOCT8 (33.86 ± 1.55, 27.34 ± 1.20, 20.61 ± 1.50 mol%), respectively. Emulsion edible films were prepared with carbohydrates, glycerol and a lipid component. There was no significant difference between SLs and HOSO and CO for water vapor permeability and density. There was a significant difference in film transparency. The HOSO film was significantly different (1.43 ± 0.27 AUmm-1), than the rest of the SLs and CO. HOCT6 (2.20 ± 0.22 AUmm-1), was significantly different than HOSO and HOCT8 (2.88 ± 0.08 AUmm-1). The use of SLs in an edible film decreases the opacity which is advantageous but mechanical properties will be tested to determine SL use in edible films.

13. **Pinolenic Acid in Structured Triacylglycerols Exhibits Superior Intestinal Lymphatic Absorption Compared to Pinolenic Acid in Pine Nut Oil** Byung Hee Kim*[1], Min-Yu Chung[2], Hyunjoon Woo[3], Juyeon Kim[4], Daecheol Kong[4], Hee-Don Choi[2], Inwook Choi[2], In-Hwan Kim[5], and Sang K. Noh[4],[1]*Sookmyung Women's University, Korea;* [2]*Korea Food Research Institute, South Korea;* [3]*Chung-Ang University, South Korea;* [4]*Changwon National University, South Korea;* [5]*Korea University, Republic of Korea*

The positional distribution pattern of fatty acids in the triacylglycerols affects intestinal absorption of these fatty acids. Pinolenic acid is a polyunsaturated fatty acid which has a blood lipid-lowering effect and acts as an appetite suppressant. This study aimed to compare lymphatic absorption of pinolenic acid present in structured pinolenic triacylglycerols (SPT) of which pinolenic acid was evenly distributed on the glycerol backbone, with absorption of pine nut oil where pinolenic acid was predominantly positioned at the *sn*-3 position. SPT were prepared via the nonspecific lipase-catalyzed esterification of glycerol with free fatty acids obtained from pine nut oil. Lymphatic absorption of pinolenic acid from pine nut oil and from SPT was compared in a rat model of lymphatic cannulation. Greater amounts of pinolenic acid were detected in lymph collected from an emulsion containing SPT than from an emulsion containing pine nut oil, thereby indicating that pinolenic acid present in SPT has a greater capacity for lymphatic absorption than pinolenic acid from pine nut oil.