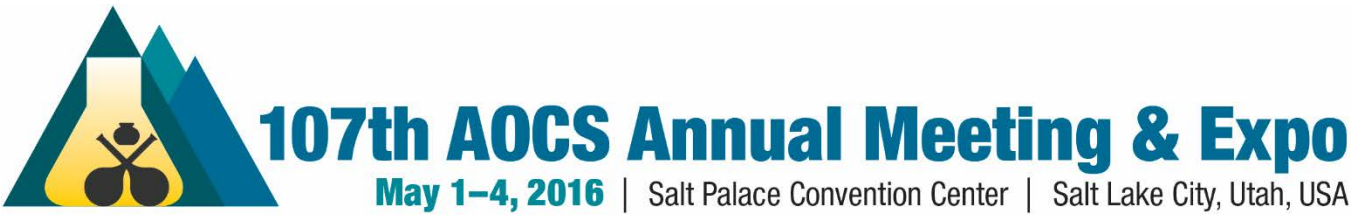

Biotechnology Interest Area
Technical Program Abstracts

Table of Contents

The presenter is the first author or otherwise indicated with an asterisk (*).
Abstract content is printed as submitted.

BIO 1: Biocatalysis I

This session is sponsored in part by Malaysian Palm Oil Board and Nisshin OilliO Group, Ltd.

Chairs: C.T. Hou, USDA, ARS, NCAUR, USA; and J. Ogawa, Kyoto University, Japan

Production of Polyol Oils from Soybean Oil by *Pseudomonas aeruginosa* E03-12. C.T. Hou[1], J.T. Lin[2], and K.J. Ray[1], [1]USDA, ARS, NCAUR, USA, [2]USDA, ARS, WRRC, USA.

Soy-polyols are important starting materials for the manufacture of polymers such as polyurethane. We have been trying to develop a bioprocess for the production of polyol oils directly from soybean oil. We reported earlier the polyol products produced from soybean oil by Acinetobacter haemolyticus A01-35 (NRRL B-59985) (Hou and Lin, 2013). In our continuous screening, we found another culture Pseudomonas aeruginosa E03-12 NRRL B-59991 which produced more polyol oils. We identified the products polyol oils as 41 derivatives of DAG, among them 32 molecular species containing one hydroxy FA and one normal FA, eight molecular species containing two hydroxy FA without normal FA, and one molecular species containing two normal FA without hydroxylated FA. The hydroxy FA included mono-, di-, and tri-hydroxy FA. Eight molecular species of DAG containing one trihydroxy FA and 14 molecular species of DAG containing one dihydroxy FA. We have also identified 64 molecular species of TAG, among them 13 molecular species containing two hydroxy FA, 42 molecular species containing one hydroxy FA, and nine molecular species containing no hydroxylated FA. This is different from our previous findings with Acinetobacter haemolyticus A01-35 which produced only DAG polyol oils.

Novel Microbial Steroid 11-beta Hydroxylase Useful for Glucocorticoid Synthesis. T. Kimura[1], N. Yoda[2], Y. Yamamoto[2], Y. Fujita[2], T. Sakamoto[2], M. Hibi[3], and J. Ogawa*[1], [1]Div. Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan, [2]Mitsubishi Chemical Group Science & Technology Research Center, Inc., Japan, [3]Lab. of Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Japan.

Microbial steroid 11-beta hydroxylation has been applied to the production of pharmaceutically important steroid derivatives. For example, hydrocortisone is produced by microbial 11-beta hydroxylation of 17-alpha,21-dihydroxypregn-4-ene-3,20-dione (compound S or 11-deoxycortisol). In this paper, we describe the identification of the novel microbial steroid 11-beta hydroxylase. The genome of Curvularia lunata MCI1688 with high steroid 11-beta hydroxylase activity was sequenced. Based on the sequence similarity to that of fungal steroid 11-alpha hydroxylase, some candidate genes were selected. Among them, two genes (P450A1 and P450A3) showed enhanced expression in RT-PCR analysis under the culture conditions inducing the steroid 11-beta hydroxylation activity of C. lunata MCI1688. These two genes were cloned and expressed in Saccharomyces cerevisiae. In the reaction with compound S as the substrate, S. cerevisiae transformant expressing P450A1 produced hydrocortisone, indicating that P450A1 encode the steroid 11-beta hydroxylase. The S. cerevisiae transformant expressing P450A1 showed higher level of the activity than the S. cerevisiae transformant expressing mammalian 11-beta hydroxylase, CYP11B1. Characteristics of the gene product expressed in yeast are also reported.

Modification of the Number of Ester Groups in the Marketed Sucrose Fatty Acid Esters by Lipase Reactions. Y. Nishiyama, T. Aibara, H. Uehara, and Y. Ueda, Nisshin OilliO Group, Ltd., Japan.

Sucrose fatty acid ester (SE) has various features depending on its degree of esterification. For example, diester has a good property that lamellar liquid crystal can be easily formed and O/W emulsion can be made stable. On the other hand, SE available in the market is generally made by chemical synthesis methods, so it is known that the number of fatty acids esterified to a sucrose molecule varies around some central value. Actually, marketed SE has only 35 percent of diester or only 30 percent of triester at the most. Therefore, we tried to get a diester-rich or triestet-rich SE by reforming of marketed SE through the use of lipase reactions.

First, we studied various kinds of reaction systems, and then we found that when using marketed monoester-rich SE and fatty acids in tert-pentyl alcohol, *Candida antarctica* lipase was able to increase the composition ratio of diester up to about 60 percent, and *Rhizopus oryzae* lipase was able to increase the composition ratio of triester up to about 50 percent. Moreover, we found that a removal of water from the reaction mixture before and during the reaction produced an increase in the reaction rate and a further increase in the composition ratio of diester or triester. Consequently, we were able to acquire the diester-rich or triester-rich SE which contained more than 70 percent of diester or more than 60 percent of triester.

Research Advancements in Palm Oil Nutrition. Y.M. Choo, Malaysian Palm Oil Board, Malaysia.

Palm oil is the major oil produced, with annual world production in excess of 50 million tonnes. About 85% of global palm oil produced is used in food applications. Over the past three decades, research on nutritional benefits of palm oil have demonstrated the nutritional adequacy of palm oil and its products, and have resulted in transitions in the understanding these attributes. Numerous studies have demonstrated that palm oil was similar to monounsaturated oils with regards to its effects on blood lipids. Palm oil provides a healthful alternative to *trans*-fatty acid containing hydrogenated fats which have been demonstrated to have serious deleterious effects on health. The similar effects of

palm oil on blood lipids, comparable to monounsaturated vegetable oils, could very well be due to the structure of the major triglycerides in palm oil, which has an unsaturated fatty acid in the *sn*-2 position of the glycerol backbone. Recent research in animal models also showed that palm oil induced less fat deposition. Palm oil is also widely used as cooking and frying oil due to its superiority in oxidative stability. Oxidized oils are proven to be detrimental for health and palm oil still has the highest resistance towards oxidation even after long hours of continuous frying. In addition, palm oil is well endowed with a bouquet of phytonutrients beneficial to health, such as tocotrienols, carotenoids, and phytosterols. The presentation will provide an overview of studies that have established palm oil as a balanced and nutritious oil.

Using Lipidomics to Aid Understanding of How Metabolic Control Constrains Yields in Oilseed Rape. H.K. Woodfield[1], A. Cazenave-Gassiot[2], I.A. Guschina[1], M.R. Wenk[2], and J.L. Harwood*[1], [1]Cardiff University, UK, [2]National University of Singapore, Singapore.

With continuous increases in the demand for plant oils (about 5% increase per year over the last 50 years), there is an urgent need to understand how triacylglycerol (TAG) accumulation is regulated in important crops. Oilseed rape (*Brassica napus*) is the main Canadian and Northern Europe oil crop and we have been studying the control of TAG accumulation using developing embryos. In particular, we have applied flux control analysis to give quantitative measurements of the distribution of regulation in different parts of the overall pathway.

In order to gain more details of the TAG biosynthetic pathway, we have utilised sophisticated tandem mass spectrometry. This has allowed us to analyse molecular species of all the important lipid classes involved during the development of the seeds. The data have allowed us to, for example, delineate the relative importance of Kennedy pathway reactions compared to those not utilising acyl-CoA substrates. Such results will allow future informed genetic manipulation to increase oil yields.

Substrate Preferences of Recombinant Flax Long Chain Acyl-CoA Synthetases. Y. Xu[1], D. Li[1], X. Pan[1], K.M.P. Caldo[1], G. Chen[1], R. Holic[2], and R.J. Weselake*[1], [1]Dept. of Agricultural, Food, & Nutritional Science, University of Alberta, Canada, [2]Inst. of Animal Biochemistry & Genetics, Slovak Academy of Sciences, Slovakia.

Flax (Linum usitatissimum) oil is enriched in a-linolenic acid (ALA; 18:3Δ9cis,12cis,15cis) and has applications in industry and as an omega-3 edible oil. Long-chain acyl-CoA synthetase (LACS) may contribute to the enriched ALA content of flax oil. LACS catalyzes the activation of free fatty acid to form acyl-CoA, which in turn serves as an acyl-donor in glycerolipid and steryl ester biosynthesis. cDNAs representing LACS genes from the flax genome were used to transform the Saccharomyces cerevisae mutant strain BYfaa1,4Δ, which is unable to use extracellular free fatty acids for growth. Active recombinant LuLACS rescued the growth of the yeast mutant on selection media containing free fatty acid and cerulenin, a specific inhibitor of intracellular fatty acid synthesis. Over-expression of LuLACS8A, LuLACS9A or LuLAC9C resulted in an increase in neutral lipid accumulation when compared to yeast expressing empty vector. Microsomal fractions were shown contain LuLACS polypeptides based on Western blotting. *In vitro* LACS assays using different radio labeled fatty acids indicated that the three recombinant forms of LuLACS were capable of using oleic acid (18:1Δ9cis), linoleic acid (18:2Δ9cis,12cis), or ALA as substrates. LuLACS8A, however, exhibited a significantly enhanced specificity for ALA over oleic acid or linoleic acid, suggesting this enzyme form may contribute to enhanced accumulation of ALA in flax oil.

Co-production of Hydroxy Fatty Acid and Mono-rhamnolipid from Olive Oil by *Pseudomonas aeruginosa* KACC 10186. H.M. Park, S.R. Kim, I.H. Choi, J.H. Jung, and H.R. Kim*, School of Food Science & Biotechnology, Kyungpook National University, Republic of Korea.

Microbial modification of vegetable oils can often lead to special changes in their structure and biological function. Hydroxylation of fatty acid by bacterial strains is one of those modifications. Hydroxy fatty acid (HFA) is considered as important industrial material because the hydroxyl groups on fatty acid backbone give fatty acid special properties, such as higher viscosity and reactivity compared with other non-hydroxylated fatty acid. Rhamnolipids are a class of glycolipid produced by *Pseudomonas aeruginosa*, amongst other organisms, frequently cited as the best characterized of the bacterial surfactants. They have a glycosyl head group, in this case a rhamnose moiety, and a 3-(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid tail. Specifically, there are two main classes of rhamnolipids, mono-rhamnolipids and di-rhamnolipids; consisting of one or two rhamnose groups respectively. Rhamnolipids are also heterogeneous in the length and degree of branching of the HAA moiety which varies with the growth media used and the environmental conditions. In this study, we firstly report that *Pseudomonas aeruginosa* KACC 10186 can produce dihydroxxy fatty acid and mono-rhamnolipid simultaneously, and their production and ratio of two compounds can be changed necessarily according to the environmental conditions.

Production of Biodiesel from Palm Fatty Acid Distillate in a Packed Bed Reactor via Enzymatic Transesterification. J. Ryu[1,2], N.K. Choi[1,2], and I.H. Kim*[1,2], [1]Dept. of Food & Nutrition, Korea University, Republic of Korea, [2]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea.

Biodiesel was produced with palm fatty acid distillate and methanol as a asubstrate in a packed bed reactor via lipase-

catalyzed transesterification. tert-Butanol was employed as a solvent. Mixed lipase from two different type of lipases such as Lipozyme TL IM from *Thermomyces lanuginosus* and Novozym 435 from *Candida antarctica* were employed as a biocatalyst. The effect of temperature and molar ratio were investigated using a mixed lipase. Firstly, the effect of mixing ratio of two immobilized lipases was investigated and optimum mixing ratio of Lipozyme TL IM and Novozym 435 was 95:5 as weight percentage. Using the mixied lipase, optimum temperature and molar ratio of substrate (palm fatty acid distillate to methanol) were 50^{o}C and 1:6. Under the optimum condition, the maximum biodiesel yield of ca. 97% could be obtained.

Oligoglycerol Fatty Acid Esters Preparation Catalyzed by Lipase and Its Effect on the Crystallization Behavior on Diacylglycerol Oil. Y. Wang[1,2], F.L. Wan[1,2], Y.L. Ten[1,2], and A.J. Li[1,2], [1]Dept. of Food Science & Engineering, Guangdong Saskatchewan Oilseed Joint Lab., Jinan University, China, [2]Guangdong Engineering Technology Research Center for Oils & Fats Biorefinery, China.

Oligoglycerol fatty acid esters (OGEs) are an important kind of polyglycerol fatty acid esters (PGEs) which have been widely used as emulsifiers in food, medicine, and cosmetic industries. The preparation of OGEs by the esterification of oligoglycerol with linoleic acid in a solvent-free system using a lipase as the catalyst was conducted. The effects of substrate molar ratio, reaction time, reaction temperature, enzyme dosage, and water addition on the efficiency of esterification (EE) were studied. Single factor experiments and response surface methodology (RSM) were employed to optimize the reaction parameters. The reaction product was isolated by silica gel column chromatography to obtain pure mono-, di-, and triesters of diglycerol. The retardation effect of DGEs with a different esterification degree on the crystallization of DAGs was investigated using pulsed NMR spectroscopy, differential scanning calorimetry, polarized light microscopy, and X-ray diffraction. The results reveal that addition of 0.5% (w/w) DGEs could effectively retard the crystallization of high-melting DAGs by inhibiting the nucleation process and delaying the crystal growth, but their addition did not alter the crystal forms of DAGs. Moreover, the retardation effect was enhanced as the esterification degree of DGE decreased.

BIO 1.1/IOP 1/SCC 1: Biorenewable Polymers

This session developed in conjunction with the Industrial Oil Products Division and the Society of Cosmetic Chemists.

This session is sponsored in part by Soy 20/20.

Chairs: R.D. Ashby, USDA, ARS, ERRC, USA; R. Wang, CVC Thermoset Specialties-Emerald Performance Materials, USA; and T. O'Lenick, Society of Cosmetic Chemists/Surfatech Corp., USA

Novel Plant Oil-based Polymers: An Overview. D.J. Kalita[1], I. Tarnavchyk[1], S. Samanta[1], O. Shafranska[1], J. Bahr[2], A. Popadyuk[1], A. Voronov[1], D. Bajwa[3], A. Bezbaruah[4], M. Sibi[5], and B.J. Chisholm*[1,6], [1]Dept. of Coatings & Polymeric Materials, North Dakota State University, USA, [2]Research & Creative Activities, North Dakota State University, USA, [3]Dept. of Mechanical Engineering, North Dakota State University, USA, [4]Dept. of Civil Engineering, North Dakota State University, USA, [5]Dept. of Chemistry & Biochemistry, North Dakota State University, USA, [6]Materials & Nanotechnology Program, North Dakota State University, USA.

A wide variety of homopolymers and copolymers were produced from novel plant oil-based vinyl ether monomers. The monomers were produced by base-catalyzed transesterification of 2-(vinyloxy)ethanol with either a plant oil triglyceride or plant oil-derived methyl esters. By proper choice of the polymerization system, linear polymers were produced that retained the unsaturation derived from the plant oil. By preserving side-chain unsaturation, crosslinked networks were achieved either directly through autoxidation or by post-polymerization modification of the double bonds to other reactive functional groups. Select homopolymers and copolymers were found to have utility for a variety of applications including paints and coatings, shampoos, rubber compounds, and environmental remediation. The results obtained clearly show that this monomer and polymer technology provides new opportunities for the use of plant oils in industrial applications. The general concept has also been used to produce novel monomers and polymers from other renewable resources including cashew nut liquid, terpenes, and lignin.

Sucrose Octaesters as Reactive Diluents for Alkyd Coatings. A. Popadyuk[1], A. Breuer[1], J. Bahr[2], I. Tarnavchyk[3], A. Voronov[3], and B.J. Chisholm*[1,3], [1]Renuvix LLC, USA, [2]Research & Creative Activities, North Dakota State University, USA, [3]Dept. of Coatings & Polymeric Materials, North Dakota State University, USA.

Sucrose octaesters, which are 100% biobased, have been proposed as effective reactive diluents for the production of high-solids alkyd coatings. A series of five different sucrose octaesters (SOEs) derived from soybean oil methyl esters, linseed oil methyl esters, and mixtures of soybean oil and linseed oil methyl esters were synthesized and characterized. The drying-time and cured film properties of the different SOEs produced with the same drier package were determined. In general, increasing the concentration of linseed oil methyl esters used to produce the SOEs decreased drying-time, decreased viscosity, increased hardness, and decreased impact strength. The SOEs were also blended with commercial alkyd resins to produce high-solids alkyd coatings and the drying time and coating properties determined.

Agricultural Waste and Non-traditional Oil in Polyol Synthesis. C.M. Patel, A. Barot, and V. Sinha, V.P. & R.P.T.P. Science College, India.

Biomass including agricultural residues are promising alternatives to petroleum in the production of value-added products. Polyols were synthesized using a two-step process featuring polyhydric alcohols in the presence of various acid catalysts. The steps involved liquefaction of agricultural wastes followed by optimization of process parameters. Polyols were developed using different non-traditional oils to modify the liquefied products. Each polyol was characterized using both chemical and instrumental methods. Results showed that 93% of the solid raw material was converted into polyols in a PEG/Glycerin-based liquefaction system using a solid/solvent ratio of 0.25 in 60-80 minutes at 160°C. The liquefied product showed an I_{OH} of 200 to 400mg KOH/g and a viscosity of 0.93Pas. The developed polyols can be used for development of foams, adhesives, and paints. In the present study, high quality rigid polyurethane foams, commonly used as insulation materials, were developed from bio based polyols. The present work focuses on formulations, applications and property analyses of these polyols.

Shape Memory Polyurethane Elastomers from Vegetable Oils. Z.S. Petrovic and J. Milic, Pittsburg State University, USA.

A shape-memory material "remembers" its original shape and returns to its pre-deformed shape when heated. This material is an alternative to conventional actuators with applications in biomedical and other industries. Novel shape memory polyurethanes were prepared from polyols made by polymerization of hydroxynonanoic acid methyl esters generated by ozonolysis of vegetable oils. Segmented polyurethanes consisted of hard segments from MDI and butane diol and highly crystallizable soft segments of polyhydroxynonanoic acid. The materials behaved as elastomers above melting point of the soft segment and hard plastic below. Melting pointes were tunable between 40 and 60°C. The materials displayed complete recovery of shape and length when heated above the transition point.

Catalytic Copolymerization of Methyl 9,10-epoxystearate and Cyclic Anhydrides. U. Biermann[1], A. Sehlinger[3], M.A.R. Meier[3], and J.O. Metzger[1,2], [1]University of Oldenburg, Germany, [2]abiosus e.V., Germany, [3]Karlsruhe Inst. of Technology, Germany.

Plant oil derived compounds are attractive as raw material for the production of environmentally friendly and in many cases biodegradable consumer products. A great challenge is the production of polymers based on renewable feedstock. We have reported the alternating ring-opening copolymerization of methyl 9,10-epoxystearate with various cyclic acid anhydrides such as phthalic anhydride, succinic anhydride, and maleic anhydride to afford polyesters of narrow molecular weight distributions using a (salen)$Cr^{(III)}Cl$ catalyst in the presence of *n*-Bu_4NCl [1].

In this way, polyesters (M_n =2000-10000g/mol) with low glass transition temperatures were formed. The reaction is characterized by sustainable aspects, for instance, the use of starting materials derived from renewable resources (> 60%), low catalyst loadings, and no added solvent. The pending long chain alkyl groups introduced in the polyesters by the fat derived substrates attribute amorphous properties to the polymers. Various fatty epoxides are easily available and open up the possibility for the synthesis of new highly branched polyesters.

[1] U. Biermann, A. Sehlinger, M. A.R. Meier, J.O. Metzger, *Eur. J. Lipid Sci. Technol.,* DOI: 10.1002/ejlt.201400631

The Development of Polyols and Polyurethane Spray Foam from Canola and Other Prairie Oilseed Crops. J.M. Curtis[1], E. Kharraz[1], X. Kong[1], T.S. Omonov[1], Y.Y. Zhao[1], D. Treleaven[2], M. Kennedy[3], and D. Kennedy[4], [1]Lipid Chemistry Group, Dept. AFNS, University of Alberta, Canada, [2]Meadow Polymers & Consolidated Coatings, Canada, [3]Green Analytics Corp., Canada, [4]Mod Panel Inc., Canada.

The use of lipid feedstocks in chemical manufacturing is well established and these are sustainable alternatives to petrochemicals. Polyols are one such class of compounds, already commercially produced from vegetable oils. Polyols that incorporate lipids range from intact natural polyols, like castor oil, to polyols that retain certain structural elements of natural lipids, such as the triacylglyceride skeleton, and ultimately to complex polyols that little resemble the starting oil. Here we describe our development of such a range of lipid-based polyol structures with possible applications.

As an example, we have developed a polyol from canola oil, now produced at pilot scale with production facility underway. It has low viscosity and high reactivity so is suitable for use in polyurethane (PU) spray foam for building insulation. Renewable, lipid based components like this can improve the overall environmental impact over the life cycle of PU spray foam. We have developed a PU spray foam formulation with comparable properties to current commercial products, e.g. cream time, rising time, curing time, and viscosity, and so can be a direct substitute. Finally, the closed-cell content, density, mechanical properties, water absorption, and aging properties of the partly biobased foam matches existing petrochemical products.

Recent Applications of Biobased Polymer Chemistry Platforms for the Development of Novel Personal Care Ingredients. M.J. Fevola[1], F.C. Sun[1], and S.E. York[2], [1]Johnson & Johnson Consumer Inc., USA, [2]University of Oregon, USA.

Polymeric ingredients are preferred for personal care formulations due to their extraordinary combination of functionality, efficiency, and safety. As manufacturers seek to improve the sustainability of personal care products, they increasingly seek “greener” chemistry platforms for ingredients that will increase the renewable and biodegradable content in their formulations without sacrificing performance. Starch and polyglycerol (PG) are examples of versatile platforms that enable development of a broad range of functional ingredients. This presentation will discuss recent advances in the application of these chemistries for designing new amphiphilic molecules with utility as surfactants, conditioners, and thickeners. A 90% biobased, readily biodegradable polymeric surfactant was developed using alkenylsuccinate modified potato starch. This high foaming, ultra-mild cleansing agent was obtained by precisely controlling the starch molecular weight and degree of hydrophobic substitution. Modification of PG with either hydrophobic or combinations of hydrophilic and hydrophobic quaternary ammonium groups enabled the synthesis of novel compounds useful as skin and hair conditioners due to their substantivity and humectancy. PG-modified hydrophobic esters of methyl glucoside or sorbitan, e.g. dioleates, demonstrated the ability to thicken detersive surfactant systems.

Composite Feed Stocks and Imaging with Fluorescence Lifetime Microcopy. J.W. Woodcock[1], D.M. Fox[1,3], I.A. Sacui[1,2], C.S. Davis[1], and J.W. Gilman[1], [1]National Inst. of Standards & Technology, USA, [2]Georgetown University, USA, [3]American University, USA.

Design of bio-based composites is best accomplished by taking inspiration from nature. Natural composites are inherently multifunctional; often exhibiting attributes such as: strength (toughness), resilience, sensing, self-healing, light weight, degradability, and optical (camouflage, fluorescence). Designing tough composites is both important and difficult, but in natural systems it is accomplished by incorporating both intrinsic and extrinsic toughening mechanisms over multiple length scales. Molecularly flexible biopolymer matrices are often combined with nano- and micro-scale reinforcing particles or fibers. The rich functionality of bio-based components enables exquisite control of the interactions between the two phases, and allows the reinforcing phase to provide the stiffness and the matrix to provide the flexibility. Here we present examples of semi-

crystalline materials that have large potential in conventional composite applications using common thermoset and thermoplastic matrices. We show the use of basic chemistry enables modification of surface chemical potentials to improve composite interactions. In addition, with easily accessible chemistry, metrologies such as fluorescence lifetime microscopy via stimuli responsive molecular probes that were employed to monitor the stress transfer processes at the composite interphase.

Moisture Resistant Coating for Packaging Paper from Silylated Soybean Oil. C. Tambe, D. Graiver*, and R. Narayan, Michigan State University, USA.

A novel approach of silylating internal double bonds of long chain fatty acids is introduced. Hydrosilylation is so far the most popular and commercially employed way for synthesizing organosilicon compounds by grafting silanes on organic compounds by formation of Si-C bonds. Unfortunately, hydrosilylation is most effective with alkenes containing terminal double bonds. In this study, we have introduced a solvent free one step silylation process for the preparation of moisture curable silylated soybean oil. The product was characterized qualitatively by ^{1}H-NMR and ATR-FTIR, while quantified by TGA.

The moisture cure of silylated oil was studied as a function of catalyst concentration, water content, and the temperature of cure. Kraft paper, most widely used in paper packaging, was successfully coated with roll coater. It was followed by a pilot scale up of paper coating process using industrial gravure roll coaters, where more than 50,000 sq. ft. of paper was coated. Coated paper demonstrated up to 50% decrease in Cobb values and a significant decrease in the WVTR values.

BIO 2: Biocatalysis II

This session is sponsored in part by Malaysian Palm Oil Board and Nisshin OilliO Group, Ltd.

Chairs: L.K. Ju, University of Akron, USA; and M. Hosokawa, Hokkaido University, Japan

Unsaturated and Epoxy Fatty Acid Estolides Derived from Sophorolipids as Plasticizers for Poly(3-hydroxybutyrate). R.D. Ashby, D.K.Y. Solaiman, C.K. Liu, G.D. Strahan, and N.P. Latona, USDA, ARS, USA.

Unsaturated and epoxy fatty acid estolides were synthesized from ω and ω-1 hydroxy fatty acids derived from sophorolipids. These estolides were added to solution-cast poly(3-hydroxybutyrate) (P3HB) films and their plasticizing effects evaluated. Estolides caused slight reductions in melting and glass transition temperatures while crystallinity remained constant (± 2.0%). Scanning electron microscopy indicated irregular film surfaces in the presence of both estolides and the formation of pores within the film matrix. These anomalies influenced the tensile properties of the films by decreasing the tensile strength and moduli of the films and increasing their percent elongation at break. Curing the films for three months in the presence of estolide led to an enhanced tensile strength and modulus when compared to the initial films. Gamma-irradiation was applied in an attempt to crosslink the estolides; but, in fact, instigated chain scission and reduced the molecular weights by up to 81% which further reduced the tensile strength, elongation at break and modulus of the films. The tensile property deviations showed that while the tensile strength of the P3HB films declined in the presence of both estolides, the plasticity and elongation at break improved, thus validating these unique SL-derived estolides as effective plasticizers in P3HB films.

Waste Grease Conversion by Phagotrophic Algae. S. Xiao, N. Vongpanish, J. Kohl, and L.K. Ju, Dept. of Chemical & Biomolecular Engineering, University of Akron, USA.

Waste grease has high free fatty acid contents and varying chain lengths and unsaturation. They are also often present in complex mixtures with other waste materials. Their removal and reuse, particularly upgrading to higher-value, modified products of more consistent compositions, can benefit much from development of advanced technologies. We have studied the waste grease conversion by a phagotrophic algal culture. The process development and results will be presented and discussed.

Molecular Species Analysis of Tuna Oil and DHA-enriched Oils. T. Nagao[1], T. Yamada[2], S. Tanaka[1], E. Fukusaki[2], and K. Banba[2,3], [1]Osaka Municipal Technical Research Inst., Japan, [2]Osaka University, Japan, [3]Kyusyu University, Japan.

Docosahexaenoic acid (DHA) has various physiological functions, and tuna oil containing DHA and DHA-enriched oil produced by a lipase reaction from tuna oil have been used as various purposes. Because these oils are composed of many kinds of fatty acids, the molecular species of these oils have not been well understood. In this study, we identified molecular species of triacylglycerol and diacylglycerol from tuna oil (DHA, 22%) and three DHA-enriched oils (DHA, 40, 55, and 70%).

The molecular species from tuna oil were classified into three groups: (i) OOO/POO/PPO species (O, C18:1; P, C16:0) including no molecule of DHA/EPA, (ii) DPP/DPO/DOO species (D, DHA or small amount of EPA) including one molecule of DHA/EPA, and (iii) DDP/DDO species including two molecules of DHA/EPA. However, DDD species including three molecules of DHA/EPA was rarely observed in tuna oil. As the lipase reaction proceeded and DHA content increased, DPP/DPO/DOO species transferred to DDP/DDO species, and followed by the DDP/DDO species transferred to DDD species. Reaction mechanisms of the lipase reaction can be discussed from the molecular species and substrate specificity of *Candida rugosa* lipase.

Chemical Structure and Beneficial Functions of n-3 PUFA-lipids. M. Hosokawa, K. Hashimoto, and K. Miyashita, Hokkaido University, Japan.

n-3 Polyunsaturated fatty acids are well known to have health beneficial functions. Their functions are depend on chemical structure of lipids and fatty acids. In the first study, effects of n-3 PUFA-rich triglyceride (PUFA-TG) and ethylester (PUFA-EE) on lipid and glucose metabolism of diabetic/obese KK-A^y mice were examined. DHA-TG and DHA-EE decreased blood glucose level. Further, both DHA-lipids tended to decrease serum free fatty acid and neutral lipid through upregulation of hepatic ACO mRNA expression. However, their effects are not different between TG and EE from. In the second experiment, we compared the effects of EPA-TG, DPA (n-3)-TG and DHA-TG prepared by lipase on lipid metabolism on KK-A^y mice. DHA-TG significantly decreased serum free fatty acid concentration, although EPA-TG markedly decreased serum total cholesterol. Further, n-3 PUFA-TGs decreased total lipid content in the liver. DHA-TG and DPA-TG, but not EPA-TG, enhanced hepatic ACO activity to promote lipid oxidation. These results suggest that each PUFA-TG shows partly different lipid metabolism regulation depending on n-3 PUFA structure.

Improved Growth Property of Genetically Modified Pseudomonas on Crude Glycerol. D.K.Y. Solaiman and R.D. Ashby, USDA, ARS, ERRC, USA.

Pseudomonas chlororaphis is a non-pathogenic bacterium that produces an industrially valuable biodegradable polymer (i.e., poly(hydroxyalkanoate) (PHA)) and rhamnolipid (RL) biosurfactants depending on growth conditions. Crude glycerol is a coproduct stream from biodiesel production, and could serve as an economical fermentation feedstock. In this study, genetic engineering is

applied to enhance the glycerol metabolic capability of *P. chlororaphis*. The glycerol uptake facilitator gene (*glpF*) and the glycerol kinase gene *(glpK*) were PCR-cloned from *E. coli*, then inserted into a shuttle vector (pBS29P2gfp) and expressed in *P. chlororaphis* through the Pseudomonas promoter P2 on the vector. The *P. chlororaphis* recombinants expressing *glpF*, *glpK*, or *glpF* and *glpK* together were characterized with respect to cell-growth and glycerol-consumption rates in chemically defined media containing 0.5 and 1.0 % glycerol. The results showed that the expression of *glpF* and *glpK* together in *P. chlororaphis* conferred an advantageous cell growth property when grown on glycerol. Further refinement is expected to result in an improvement of the techno-economics of PHA and RL production by *P. chlororaphis* using crude glycerol coproduct stream as fermentation feedstock.

Enzymatic Modification of Anhydrous Milkfat with n-3 and n-6 Fatty Acids for Potential Use in Infant Formula: A Comparison of Methods. M.J. Sproston and C.C. Akoh, University of Georgia, USA.

A structured lipid (SL) with a high amount of *sn*-2 palmitic acid was synthesized from anhydrous milkfat and was then enriched with docosahexaenoic (DHA) and arachidonic (ARA) acids using an immobilized lipase. Three different methods were compared, including physical blending, enzymatic interesterification, and enzymatic acidolysis. Products were compared with respect to differences in fatty acid profiles, reaction times, antioxidant contents, oxidative stability, melting and crystallization profiles, and reaction yields. The acidolysis method was the least suitable for the synthesis of desired product because of a low reaction yield, low incorporation of DHA, low oxidative stability, and the extra processing steps required. The physical blending and interesterification methods were suitable, but the interesterification product (IE-SL) had higher amounts of ARA at the *sn*-2 position. The IE-SL contained total ARA and DHA of 0.63 and 0.50mol % and 0.55 and 0.46mol % at the *sn*-2 position, respectively. The IE-SL also contained 44.97mol % *sn*-2 palmitic acid. The reaction yield for the IE-SL was 91.84%, and its melting completion and crystallization onset temperatures were 43.1 and 27.1°C, respectively. This SL might be totally or partially used in commercial fat blends for infant formula.

EPA Production by an Oleaginous Fungus *Mortierella alpina* Breeding at Moderate Temperature. A. Ando[1], T. Okuda[2], H. Kikukawa[2], E. Sakuradani[3], and J. Ogawa[1,2], [1]Research Unit for Physiological Chemistry, Kyoto University, Japan, [2]Div. of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan, [3]Inst. of Technology & Science, University of Tokushima, Japan.

The oleaginous fungus *Mortierella alpina* is known to accumulate eicosapentaenoic acid (EPA) only when cultivated at a low temperature (below 15°C). In this study, we investigated EPA production at moderate temperature (28°C) by expressing the *Saprolegnia diclina* ?17 desaturase gene (*sdd17m*) in *M. alpina* ST1358, an ?3-desaturation activity-defective mutant derived from *M. alpina* 1S-4. Expression of the exogenous gene was confirmed by EPA accumulation in transformants at both 28 and 12°C. The EPA content in total lipids produced by transformants was over 20% at 28°C. Bench-scale fermentation with a 5-L jar fermentor showed that EPA content reached 26.4% of total fatty acids, and EPA production reached 1.8?g/L. This is the first study to report the accumulation of EPA in *M. alpina* at moderate temperature, and provide a platform technology for the industrial production of EPA using *M. alpina* as a promising source for EPA.

Enzymatic Characterization of Hydroxy Fatty Acid Dehydrogenas from *Lactobacillus plantarum* AKU 1009a Useful for Oxo Fatty Acid Production. S. Kishino, M. Takeuchi, and J. Ogawa, Kyoto University, Japan.

Lactic acid bacteria are very useful microorganisms as the probiotics. Recently, we revealed polyunsaturated fatty acid saturation metabolism, which consists of four enzymes, *i.e.*, CLA-HY (hydratase/dehydratase), CLA-DH (dehydrogenase), CLA-DC (isomerase), and CLA-ER (enone-reductase), in *Lactobacillus plantarum* as a model strain [1]. This metabolism generates several types of fatty acids such as hydroxy, oxo, and conjugated fatty acids. In those of metabolites, we revealed some physiological functions. For example, 10-hydroxy-*cis*-12-octadecenoic acid (HYA) has ameliorates intestinal epithelial barrier impairment. 10-oxo-*cis*-12-octadecenoic acid (KetoA) activates PPARγ and stimulates adipogenesis. In this study, we analyzed enzymatic characteristics of CLA-DH, which catalyzes reversible dehydrogenation of hydroxy fatty acids to oxo fatty acids. CLA-DH required NAD^+ and NADH as the cofactors for oxidation and reduction reactions, respectively. In the reduction reaction of CLA-DH, both enantiomers of HYA were produced from KetoA. In the oxidation reaction of CLA-DH, (*R*)-HYA was dehydrogenated with same relative activity of (*S*)-HYA. These results indicate that CLA-DH has low enantioselectivity.

[1] S. Kishino *et al.*, *Proc. Natl. Acad. Sci. USA*, **110**, 637–640 (2013).

Characterization and Heterologous Expression of a Novel Galactolipase from *Chlorella kessleri*. D. Sugimori[1], K. Fujiuchi[1], S. Hashiro[2], and H. Yasueda[2], [1]Fukushima University, Japan, [2]AJINOMOTO Co. Inc., Japan.

Lysogalactolipid and galactosylglycerol attract attention as interesting applications in foodstuffs, cosmetic, and health-care products. We have found an enzyme, galactolipase (chGL), which hydrolyzes the acyl ester bond of galactolipids such as digalactosyldiacylglycerol (DGDG), from *Chlorella kessleri*. Here we report characterization, gene cloning, and heterologous expression of chGL.

chGL was calcium ion-dependent and monomeric protein (53kDa). The substrate specificity was in the order: DGDG (100%)>monogalactosyldiacylglycerol=phosphatidylglycerol (~40%)>sulfoquinovosyldiacylglycerol (~20%). The maximum activity was found at pH 6.5 and 37°C. The cDNA for chGL was comprised of a coding sequence of 1530bp encoding a 475-amino-acid (aa) protein with a 35-aa signal sequence. The deduced aa sequence of chGL showed no significantly similarity with GLs of *Chlamydomonas* and *Aspergilus*. Recombinant chGL was produced by *Brevibacillus/*pBIC and *E. coli*/pCold. GC analysis demonstrated that chGL preferably hydrolyzed the *sn*-1 acyl ester bond (*sn*-1/-2 ratio of 9:1). Structure modeling and mutational analyses revealed that the active residues would be Ser271, Asp347, and His440. We believe chGL can be useful as food-processing enzyme such as quality improvement of wheat flour and its products.

BIO 3: Biomodifications, Biomechanisms, Biosafety

Chairs: S.H. Yoon, Woosuk University, Republic of Korea; and T. Sugawara, Kyoto University, Japan

Production of Hydroxy Fatty Acids with Health Promoting Activity Using Linoleic Acid Hydratase Involved in Polyunsaturated Fatty Acid Saturation Metabolism in Lactic Acid Bacteria. M. Takeuchi, S. Kishino, S.B. Park, N. Kitamura, and J. Ogawa, Kyoto University, Japan.

Hydroxy fatty acids exert various physiological effects supporting our health. Recently, we revealed polyunsaturated fatty acid saturation metabolism in gut microorganisms with *Lactobacillus plantarum* as a model strain (1). This metabolism generates several types of fatty acids such as hydroxy, oxo, and conjugated fatty acids. Furthermore, we revealed that (*S*)-10-hydroxy-*cis*-12-octadecenoic acid (HYA), an intermediate in the metabolism, has immunomodulatory activity (2) and ameliorates intestinal epithelial barrier impairment (3). In this study, we tried to produce hydroxy fatty acids using CLA-HY, which is the hydratase involved in the saturation metabolism. HYA was produced from linoleic acid (1M, 280g/L) with high conversion rate of 98% (mol/mol) and more than 99.9% *e.e.* under optimum reaction conditions with *E. coli* expressing CLA-HY. Other C18 Δ9 unsaturated fatty acids such as oleic acid, α-linolenic acid, and γ-linolenic acid (280g/L) were converted to corresponding 10-hydroxy fatty acids with conversion rates of over 95%. The various hydroxy fatty acids provided by this technology are useful for further application for developing novel functional fatty acids.

1 S. Kishino *et al PNAS* **110** 637–640, 2013
2 P. Bergamo *et al J Funct Foods* **11** 192–202, 2014
3 J. Miyamoto *et al JBC* **290** 2902–2918, 2015

Hypolipidemic Effect of Gut Microbial Fatty Acid Metabolites in Hepatocytes. T. Nanthirudjanar[1], H. Furumoto[1], J. Zheng[1], Y.I. Kim[1], T. Goto[1], N. Takahashi[1], T. Kawada[1], S.B. Park[1], A. Hirata[1], N. Kitamura[1], S. Kishino[1], J. Ogawa[1], T. Hirata[1,2], and T. Sugawara*[1], [1]Kyoto University, Japan, [2]Shijonawategakuen University, Japan.

Lactobacillus plantarum, a non-pathogenic gram-positive bacterium that naturally exists in gastrointestinal tracts of humans, can produce various hydroxy and oxo fatty acids from unsaturated fatty acids. In this study, we investigated the effects of these gut microbial fatty acid metabolites on the lipogenesis in liver cells. We focused on the effect on sterol regulatory element-binding protein-1c (SREBP-1c) expression in HepG2 cells treated with synthetic liver X receptor a (LXRa) agonist (T0901317). We found that 10-hydroxy-*cis*-12-octadecenoic acid (18:1) (HYA), 10-hydroxy-*cis*-6,*cis*-12-octadecadienoic acid (18:2) (?HYA), 10-oxo-*cis*-12-18:1 (KetoA), and 10-oxo-*cis*-6,*cis*-12-18:2 (?KetoA) significantly decreased SREBP-1c mRNA expression induced by an LXRa agonist. These four types of fatty acids also down-regulated the mRNA expression of lipogenic genes via the antagonism of LXRa and the inhibition of SREBP-1 maturation. In addition, oral administration of KetoA for two weeks significantly decreased the mRNA expression of SREBP-1c, stearoyl-CoA desaturase-1 (SCD-1), and acetyl-CoA carboxylase-2 (ACC2) in the liver of high sucrose diet-fed mice. Our findings strongly suggest that hypolipidemic effect of the fatty acid metabolites produced by *L. plantarum* can be taken advantage of in the treatment of cardiovascular diseases or dyslipidemia.

A Gut Microbial Fatty Acid Metabolite, 10-Oxo-*trans*-11-Octadecenoic Acid, is Cytoprotective Against Oxidative Stress. H. Furumoto[1], T. Nanthirudjanar[1], T. Kume[1], Y. Izumi[1], S.B. Park[1], N. Kitamura[1], S. Kishino[1], J. Ogawa[1], T. Hirata[1,2], and T. Sugawara*[1], [1]Kyoto University, Japan, [2]Shijonawategakuen University, Japan.

In this study, we investigated the effects of novel fatty acid metabolite derivatives of linoleic acid generated by the gut lactic acid bacteria *Lactobacillus plantarum* on the nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) pathway, which plays a central role in cellular antioxidative responses. As the result of cultured cell study, 10-oxo-*trans*-11-octadecenoic acid (KetoC) most effectively protected HepG2 cells from cytotoxicity induced by hydrogen peroxide among the gut microbial fatty acid metabolites. KetoC also significantly increased cellular Nrf2 protein levels, ARE-dependent transcription, and the gene expression of antioxidative enzymes, such as heme oxygenase-1 (HO-1), glutamate-cysteine ligase modifier subunit (GCLM), and NAD(P)H:quinone oxidoreductase 1 (NQO1) in HepG2 cells. Additionally, an oral dose administration of KetoC also increased antioxidative gene expression and protein levels of Nrf2 and HO-1 in mouse organs. This cytoprotective effect of KetoC may be due to its a,ß-unsaturated carbonyl moiety. Our data indicates that KetoC activated the Nrf2-ARE pathway to enhance cellular antioxidative responses and may prevent multiple diseases induced by oxidative stress.

Molecular Functions of a Linoleic Acid Metabolite Produced by Gut Lactic Acid Bacteria for Obesity-related Metabolic Diseases. T. Goto[1,2], Y.I. Kim[1], T. Furuzono[1], K. Yamakuni[1], N. Takahashi[1,2], T. Sugawara[1], N. Kitamura[1], S.B. Park[1], S. Kishino[1], J. Ogawa[1,2], and T. Kawada[1,2], [1]Graduate School of Agriculture, Kyoto University, Japan, [2]Center for the Promotion of Interdisciplinary Education & Research, Kyoto University, Japan.

Gut microbiota alterations exert profound effects on host physiology and metabolism, as well as obesity and obesity-related diseases. Here we show that functions of a linoleic acid (LA) metabolite produced by gut lactic acid bacteria for obesity-related metabolic diseases. We showed that two fatty acids from LA potently activated PPAR?, a master regulator of adipocyte differentiation, with 10-oxo-12(Z)-octadecenoic

acid (KetoA) having the most potency. In 3T3-L1 cells, KetoA induced adipocyte differentiation via the activation of PPAR?, and increased adiponectin production and insulin-stimulated glucose uptake. Moreover, obese diabetic mice fed KetoA-containing diet showed reduction in white adipose tissue accumulation accompanied with the improvement of obesity-related metabolic dysfunctions. Uncoupling protein 1 (UCP1), a key molecule in nonshivering thermogenesis in brown adipose tissue, expression and oxygen consumption rate in mice fed KetoA were increased, suggesting that KetoA increased energy expenditure via the up-regulation of UCP1 function in adipose tissues. We are now addressing molecular mechanisms underlying KetoA-induced amelioration for obesity and obesity-induced metabolic diseases. We will discuss more details of KetoA functions in obese model mice.

The Intestinal Barrier Recovering-function of a Gut Microbial Metabolite of Linoleic Acid. J. Miyamoto[1,2], S. Kishino[3], T. Suzuki[1], J. Ogawa[3], I. Kimura[2], and S. Tanabe[1], [1]Graduate School of Biosphere Science, Hiroshima University, Japan, [2]Graduate School of Agriculture, Tokyo University of Agriculture & Technology, Japan, [3]Graduate School of Agriculture, Kyoto University, Japan.

Gut microbial metabolites of polyunsaturated fatty acids have attracted much attention because of their various physiological properties [(1)]. Dysfunction of tight junction (TJ) in the intestine contributes to the pathogenesis of many disorders such as IBD. We evaluated the effects of gut microbial metabolites on TNF-a-induced barrier impairment in Caco-2 cells and DSS-induced colitis in mice. 10-Hydroxy-*cis*-12-octadecenoic acid (HYA), a gut microbial metabolite of linoleic acid, suppressed TNF-a, and dextran sulfate sodium-induced changes in the expression of TJ-related molecules. HYA also suppressed TNF receptor 2 (TNFR2) expression in Caco-2 cells and the inflamed colonic tissue. In addition, HYA suppressed the protein expression of TNFR2 in murine intestinal epithelial cells. Furthermore, HYA induced $[Ca^{2+}]i$ responses in HEK293 cells expressing human GPR40 with higher sensitivity than linoleic acid, its metabolic precursor. The barrier-recovering effects of HYA were abrogated by a GPR40 antagonist and MEK inhibitor in Caco-2 cells. Therefore, HYA modulates TNFR2 expression, at least partially, via the GPR40-MEK-ERK pathway and may be useful in the treatment of TJ-related disorders such as IBD [(2)].

(1) Kishino *et al.*, 2013. *Proc Natl Acad Sci U S A.* 110 (44): 17808-17813.

(2) Miyamoto *et al.*, 2015. *J Biol Chem.* 290 (5): 2902-2918.

Lymphatic Absorption and Body Fat Reduction of Structured Conjugated Linoleic Triacylgycerols in Murine Models. M.Y. Chung[1], H.D. Choi[1], S.K. Noh[2], and B.H. Kim[3], [1]Korea Food Research Inst., Republic of Korea, [2]Changwon National University, Republic of Korea, [3]Sookmyung Women's University, Republic of Korea.

This study aimed to examine the lymphatic absorption capacity and body fat reduction effect of structured triacylglycerols enriched in conjugated linoleic acid (CLA-TAG) in murine models. CLA-TAG (containing 70.3% CLA) was prepared via a lipase-catalyzed esterification of glycerol with commercial CLA mixtures (CLA-FFA). Lymphatic absorption of CLA-TAG and CLA-FFA was compared in a rat model of lymphatic cannulation. Higher amounts of CLA were detected in the collected lymph from lipid emulsion containing CLA-TAG. Body fat reduction effect of CLA-TAG at different levels was examined using diet-induced obese mice. High fat diet (HFD) feeding for 12 weeks causes significant increase in body weight and epididymal and retroperitoneal fat masses, which were significantly decreased by dietary supplementation of 2% CLA-TAG. However, CLA-TAG at 2% caused hepatomegaly. Meanwhile, CLA-TAG at 1% significantly reduced retroperitoneal fat mass. Furthermore, the weight of livers from HFD with 1% CLA-TAG was statistically no different with that of normal diet controls. Collectively, CLA-TAG has greater lymphatic absorption capacity than CLA-FFA and CLA-TAG at 1% supplementation reduces retroperitoneal fat mass without apparent hepatomegaly, known side-effect of CLA in mouse model of obesity.

Production of Monoacylglycerol via Enzymatic Glycerolysis Using Novel Esterase in Reversed Micellar Solvent System. S.H. Yoon[1] and P.S. Chang[2], [1]Woosuk University, Republic of Korea, [2]Seoul National University, Republic of Korea.

Esterase (carboxyl esterase. carboxyl-ester hydrolase; EC 3.1.1.1) is an enzyme producing free fatty acids, mono- and di-acylglycerols from tri-acylglycerols. *Calotrophis procera* (Asclepiadaceae) R. Br. is an evergreen tree with wide and large leaves growing in tropical regions of Asia, Africa, Australia, and surrounding regions. It has been traditionally been utilized as a herbal medicine in the regions. Estrase was obtained from *C. procera*, purified, and characterized. Two kinds of estrases were isolated and purified. Total esterase activity obtained was almost same as that of commercial products. Midium-chanin monoacylglycerols were produced from glycerol and capric acid using estrase in AOT-isooctane reversed micellar system. Reaction conditions were optimized in terms of surfactant type, organic solvent, G-value, R-value, pH, temperature, reaction time, and effects of metal ions.

Pancreatic Lipase-related Protein 2 as Efficient Tool for Bioconversion of Galactolipids into Fatty Acid Alkyl Esters. J. Lecomte[1], S. Amara[2], N. Barouh[1], D. Lafont[3], J.D. Rodier[4], M. Arnaud[4], F. Demarne[4], P. Villeneuve*[1], and F. Carrière[2], [1]CIRAD, UMR IATE, France, [2]CNRS, Aix-Marseille Université, France, [3]Inst. de Chimie et Biochimie Moléculaires et Supramoléculaires, France, [4]Gattefossé SA, France.

Mono- and di-galactosyldiacylglycerol (MGDG, DGDG) from plant chloroplasts are the main but unexploited source of fatty acids (80%) on earth. Although plant galactolipases (galactolipid acyl hydrolases) are known for a long time, the

existence and role of pancreatic lipase-related protein 2 (PLRP2) in the gastrointestinal tract have been highlighted only from the mid-90s. Recently, we demonstrated that recombinant PLRP2 from guinea pig (rGPLRP2) efficiently hydrolyzed medium and long chain MGDG and DGDG. Here, we reported the potentialities of this rGPLRP2 and a cutinase from *Fusarium solani pisi* as biocatalysts for *in situ* conversion of spinach leaves galactolipids into free fatty acids (FFA) and their alkyl esters (FAAE). Alcoholysis was carried out on fresh leaves in buffered alcohols (pH8, 37°C) with an enzyme load of 1.0-1.5mg/g spinach. The best results were obtained with rGPLRP2 that totally converted MGDG and DGDG in less than 8h at optimal concentrations of 6M and 2.5M for methanol and ethanol respectively. Despite the competitive hydrolysis of MGDG and DGDG into fatty acids (up to 16x faster than that of alcoholysis), a high molar ratio FAAE/FFA=2 was observed with both alcohols. Finally, whatever the enzyme, none reaction occurred in buffered glycerol or pure alcohols.

BIO 3.1/IOP 3: Biofuels I

This session developed in conjunction with the Industrial Oil Products Division.
Chairs: H.C. Holm, Novozymes A/S, Denmark; and G. Knothe, USDA, ARS, NCAUR, USA

Diesel R33. J. Krahl[1,2], K. Götz[1], B. Fey[3], and J. Bünger[4,2], [1]Technology Transfer Center Automotive of Coburg University (TAC), Germany, [2]Fuels Joint Research Group, Germany, [3]Thünen-Inst., Germany, [4]Research Inst. for Prevention & Occupational Medicine of the German Social Accident Insurance, Ruhr University Bochum (IPA), Germany.

Greenhouse gas (GHG) reduction is a key driver for new technologies in the European Union but also in other countries of the world. Drop-in biofuels can contribute to that goal. The new developed biofuel Diesel R33 enables sustainable mobility fulfilling the European diesel fuel specification.

Diesel R33 is made from 7% used cooking oil methyl ester, 26% hydrotreated vegetable oil (HVO), and 67% high quality diesel fuel. HVO was produced from rapeseed and palm oil.

Diesel R33 was tested in a fleet of 280 vehicles (passenger cars, light duty vehicles, and buses) covering all emission classes. The impact of the new fuel on vehicles, emissions, and the engine oils was investigated.

At the example of five passenger cars (Euro 3, 5, and 6) regulated and non-regulated exhaust emissions were determined.

All vehicles fulfilled the exhaust regulations for both fuels. Except NO_x, Diesel R33 showed a positive impact on regulated and non-regulated emissions, such as carbonyls and polycyclic aromatic hydrocarbons (PAH). Moreover, the mutagenic effects of the exhaust gases were measured. At Euro 5 and 6 passenger cars, PAH and mutagenicity were hardly detectable for both fuels. The positive effects were expressed more clearly at older engine technology.
Most relevant is the GHG reduction of 17% versus fossil diesel fuel.

In addition, two vehicles were successfully powered by Diesel R33 based on algae and yeast oil.

In all, 1.900.000 liter Diesel R33 were used without any problems.

Unique Lipids from a Common Algae: Investigating the Biofuel Potential of Commercial *Isochrysis*. G.W. O'Neil[1], G. Knothe[2], and C.R. Reddy[3], [1]Western Washington University, USA, [2]USDA, ARS, USA, [3]Woods Hole Oceanographic Institution, USA.

Isochrysis is one of a select number of algae currently grown industrially, harvested for purposes of mariculture, and available in multi-kilogram quantities from several suppliers worldwide. It is also one of only a few taxonimically restricted algae that in addition to traditional fatty acids, biosynthesize significant quantities of a unique suite of lipids known as polyunsaturated long-chain alkenones. Results from the parallel production of two liquid fuel streams utilizing both fatty acids and alkenones from commercial *Isochrysis* are presented. These materials were produced on sufficient scale to allow for comprehensive testing according to ASTM standards. The isolation of other valuable coproducts such as medicinal carotenoids to potentially offset biofuel production costs is also described.

Low-temperature Phase Behavior of Fatty Acid Methyl Esters by Differential Scanning Calorimetry (DSC). R.O. Dunn, USDA, ARS, NCAUR, USA.

Fatty acid methyl ester (FAME) mixtures have many uses including biodiesel, lubricants, metal-working fluids, surfactants, polymers, coatings, green solvents, and phase-change materials. The physical properties of a FAME mixture depend on the fatty acid concentration (FAC) profile. Some products have high concentrations of saturated FAME and other components with high melting points that can crystallize at moderate temperatures. Liquid-solid (L-S) phase transitions, both melting and crystallization, can be efficiently analyzed by differential scanning calorimetry (DSC). This paper evaluates the use of DSC to measure onset, peak maximum and offset temperatures, and enthalpies of fusion of pure FAME. Furthermore, the subsequent use of the DSC data in thermodynamic models to determine crystallization onset and other phase transition temperatures that take place in FAME mixtures is discussed. Finally, the limitations of DSC analysis of FAME mixtures, such as supercooling and polymorphism, are explored.

An Overview of the Properties of Fatty Acid Alkyl Esters. G. Knothe, USDA, ARS, NCAUR, USA.

Fatty acid alkyl esters of plant oils, especially in form of methyl esters, have numerous applications with fuel use having received the most attention in recent times due to the potential high volume. Various properties imparted by neat fatty acid alkyl esters have been shown to influence fuel use, including not only cetane number and other combustion-related aspects, but also kinematic viscosity, melting point, oxidative stability, lubricity, and density. These properties, however, are of significance also for other applications of fatty acid alkyl esters, for example, lubricants and solvents. An overview of these properties will be given in relation not only to fuel use but also in relation to other applications.

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

Residual free glycerin (FG) is a critical marker of biodiesel (B100) quality because of well known deleterious effects of excessive concentrations. Yet, routine determinations by standard methods are well known to display considerable imprecision, which undermines the value of the data. We present the results of a systematic investigation of day to day variations in FG determinations of 13 B100 samples. Elevations caused by vigorous agitation in certain samples indicate the existence of heterophases with limited solubility, and represent a potential source of imprecision and severe sampling bias, previously undocumented. Moreover, bias is observed at levels of FG initially near or below 0.02% in polished B100 samples. We find the solubility of FG in dry soy biodiesel is much lower than expected based upon earlier studies, which helps explain the results. Further, our data indicate that dissolved moisture residuals can interact, sharply reducing the solubility of FG to levels below internationally recognized limits.

Both the magnitude of imprecision and bias are greatly improved by a promising sample pretreatment which is both readily implemented and compatible with standard GC silyl methods. These results and their implications relative to the fate of FG residuals in B100 parcels are discussed.

Enzymatic Conversion of Brown Grease to Biodiesel Fuel. S. Basheer, TransBiodiesel Ltd., Israel.

The use of lipases as an alternative for conventional chemical catalysts has been intensively evaluated for the esterification/transesterification of oils and fats with methanol for the production of fatty acid methyl esters to be used as biodiesel fuel. Different lipases either in their native or immobilized forms have been tested, including those derived from *Candida antarctica B, Alcaligenes sp., Pseudomonas cepacia, Thermomyces lanuginose,* and *Candida rugosa.* Reported results showed that most lipases used so far have been inhibited by the different components present in the reaction medium, e.g. methanol, FFA's, and water and glycerol produced as byproducts of the esterification/transesterification reactions. This work will show for the first time the use of a new immobilized lipase for the conversion of low-quality feedstocks, such as brown grease to biodiesel complying with the ASTM spec. The tolerance degree of the immobilized lipase to methanol, water and glycerol, will also be demonstrated in this work. Other parameters of potential effect on the conversion rates, such as temperature, FFAs content, and amount of biocatalyst will also be presented.

New Enzymatic Concept in Crude Oil Transesterification. P.M. Nielsen, Novozymes A/S, Denmark.

A large volume of biodiesel production is based on crude oil for instance soybean oil or canola oil. Before the oil is reacted in the sodium methoxide catalyzed transesterification it is refined to eliminate FFA and phospholipids. Standard procedure for the refining step is a caustic washing step followed by separation. This refining step leads to significant yield loss dependent on the amount of FFA and phospholipids in the crude oil. One way of reducing yield loss is enzymatic degumming either using a phospholipase C or phospholipase A. But even with this improvement there will still be some yield loss in the separation step and due to the content of FFA.

We have found that it is possible to decrease the yield loss significantly in the biodiesel process if the enzymatic degumming process is merged with enzymatic transesterification and carried out simultaneously. The separation step is eliminated and the FFA in the crude oil as well as FFA and glycerides produced from the phospholipase reaction will be esterified in the enzymatic process. This leads to a significant overall total yield increase. The data from the optimized process will be discussed to document the new combined degumming-transesterification process.

Enzymatic Biodiesel—Single Time Use of Enzyme and One Pot Polishing Solution. A. Rancke-Madsen, P.M Nieslen, H.C. Holm, T. Balle, and M.K Bollinger, Novozymes A/S, Denmark.

The use of liquid enzymes in biodiesel production has been a break-through as liquid enzymes can handle 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 pot process”. The new process has been tested in both lab scale and large scale and has proven superior to the current process due to simplicity, lower process variations, and excellent separation performance. Reaction conditions are 2-3kg enzyme/ton oil, 1-2% water, 1.4-1.8 equivalents of methanol and 24-36 hours of reaction time at 95-113°F/35-45°C. After reaction, dilute caustic is mixed in to the reaction mixture at 130°F/60°C for 30 minutes. After a few hours of settling time, the FAME phase is separated from the heavy phase, washed with 2% water and dried. The FAME yield is 96% and the glycerol heavy phase has low salt content. A few options for optimizing the yield closer to 100% will be discussed.

BIO 4: Plant Lipid Biotechnology and Genomics

This session sponsored in part by Alberta Innovates Phytola Centre.

Chairs: R.J. Weselake, University of Alberta, Canada; and J.L. Harwood, Cardiff University, UK

Improved Chia Production and Development of NIRS Calibrations. D.F. Hildebrand, University of Kentucky, USA.

Being among the best sources of ω3 fatty acids, healthful fiber as well as an excellent source of protein and calcium the demand for chia, *Salvia hispanica*, as a whole food and food ingredient continues to grow. To improve agronomic properties top yielding regular short-day lines were crossed with our most promising long-day flowering chia lines. One of our most promising long-day flowering chia lines was mutagenized and it is being selected for lines with larger seeds, seeds with higher oil content, and lower shattering and lodging. We have found some new mutants with 79% larger seed size. One of our most promising chia lines averaged 1.4 mg/seed. New selected lines have been found to average 2.5 mg/seed. A collection of chia seeds from around the world was screened for oil, fatty acid and protein and included in development of NMR and NIRS calibrations. Preliminary analysis indicates that chia has a very small genome, 2C ~ 0.9 pg. The long-day flowering trait is recessive and is inherited as a single gene. This will greatly facilitate breeding long-day flowering chia.

Characteristics of Arabidopsis GPAT9. G. Chen[1,2], S.D. Singer[1], E. Mietkiewska[1], P. Tomasi[3], K. Jayawardhane[1], J.M. Dyer[3], and R.J. Weselake[1], [1]Dept. of Agricultural, Food, & Nutritional Science, University of Alberta, Canada, [2]Dept. of Biological Sciences, University of Manitoba, Canada, [3]USDA, ARS, Arid-Land Agricultural Research Center, USA.

Arabidopsis has six ER-localized glycerol-3-phosphate acyltransferases (GPATs 4-9). GPATs 4-8 have been shown to be involved in the production of surface lipid polyesters. Although Arabidopsis GPAT9 was recently reported to be essential for triacylglycerol biosynthesis in developing Arabidopsis seeds by our group and other researchers (Singer et al., Botany 2015, July 24-30, Edmonton, Canada; Singer et al., the 11th International Symposium on Biocatalysis and Agricultural Biotechnology, 2015 September 13-16, Banff, Canada; Shockey et al., Plant Physiology, 2016, 170:163-79), the function of this GPAT in the biosynthesis of polar lipids and triacylglycerols in non-seed organs, as well as in surface lipids, has not been investigated. In this study, we demonstrate that Arabidopsis GPAT9 contributes to intracellular glycerolipid biosynthesis in developing leaves and pollen grains but has no function in surface lipid biosynthesis. Rather than creating a supply of sn-2 monoacylglycerol to fuel the biosynthesis of extracellular polyesters, we show that GPAT9 exhibits sn-1 acyltransferase activity with high specificity for acyl-coenzyme A, which is consistent with the role of the enzyme in the sn-glycerol-3-phosphate pathway leading to polar lipid and triacylglycerol production.

Non-GMO Tilling for Oil Traits. D. Facciotti, I. Dicely, D. Loeffler, C. McGuire, and J. Goodstal, Arcadia Biosciences, USA.

TILLING is now a mature, 15 years old technology. It has become quite popular in recent years not only for for research purposes, in helping determining the role of given genes, but also in the development of commercial products. In the latter case it takes advantage of the lack of onerous regulatory hurdles that usually hinder the development of GMO products. It can therefore address traits typically associated with “smaller” products, especially quality traits that would not be affordable *via* GMO. New technologies associated to gene transfer such as CRIPR/Cas can compete with TILLING and we will spend a few minutes comparing the two technologies. Mainly, however, we will present TILLING applications addressing soybean oil traits, both qualitative, as affecting oil composition, or quantitative as affecting oil content.

Spatial Distribution of Lipid Molecular Species During Oil Accumulation in Developing Oilseed Rape Embryos. H.K. Woodfield[1], D. Sturtevent[2], I.A. Guschina[1], K.D. Chapman[2], and J.L. Harwood*[1], [1]Cardiff University, UK, [2]University of North Texas, USA.

Oilseed rape (*Brassica napus*) is the third most important source of plant oil. Its oil, which is high in oleate, has uses not only for edible purposes but also as a renewable source of chemicals and biofuel. It is the major oil crop grown in Canada and Northern Europe.

We have been studying the regulation of oil accumulation in oilseed rape, in particular by utilising flux control analysis to identify important control points in biosynthesis. As part of this study, we have utilised MALDI-MS imaging in order to reveal more detail about the formation of triacylglycerol (TAG).

We have used embryos at 20, 27 and 35 days after flowering. This represents the beginning, mid and the end of the rapid phase of oil accumulation. The data reveal striking differences in the distribution of individual molecular species for separate lipid classes. The results provide additional information to reveal major regulatory pathways for TAG production. They also show unexpected subtleties in the capacity of different parts of the seed to synthesise complex lipids.

The Challenge of Making Hydroxy Fatty Acids in Transgenic Plants. T.A. McKeon, USDA, ARS, WRRC, USA.

The castor oil plant produces a seed containing up to 60% oil containing 85-90% ricinoleic acid, 12-hydroxy oleic acid. The presence of the mid-chain hydroxyl group imparts physical and chemical properties making castor oil uniquely

useful as a feedstock for numerous products. The seed protein ricin is a significant impediment to widespread acceptance of castor as a cultivated crop. As a result, there has been great interest in developing a transgenic crop that produces ricinoleate or other hydroxy fatty acids (HFA). The challenge in understanding how the castor plant makes such a unique oil has been confronted by biochemists and plant molecular biologists for over 50 years. Early investigations identified the oleoyl hydroxylase activity. Eventual cloning of the hydroxylase and expression of the gene in a transgenic plant resulted in <20% HFA. Since the first transgenic expression of the hydroxylase, a number of enzymes involved in castor oil biosynthesis have been cloned and tested in model plants. At this time, the highest level of HFA achieved is 33%. This presentation will describe our results and review results of other researchers to describe the roadblocks to producing an oil with high levels of HFA. At this time, it appears that a combination of evolutionary differences and competitive reactions can account for the difficulty in developing a transgenic replacement for castor oil.

Metabolic Engineering and Field Production of Camelina that Accumulate Acetyl-TAG Oils with Reduced Crystallization Temperature, Viscosity, and Calories. J.B. Ohlrogge[1,2], J. Liu[1,2], R.L. Evangelista[3], T.A. Isbell[3], M.J. Pollard[1,2], and T.P. Durrett[4], [1]Dept. of Plant Biology, Michigan State University, USA, [2]Great Lakes Bioenergy Research Center, USA, [3]USDA, ARS, NCAUR, Bio-Oils Research Unit, USA, [4]Dept. of Biochemistry & Molecular Biophysics, Kansas State University, USA.

The seeds of Burning Bush (*Euonymus alatus*) produce acetyl-TAG, a triacylglycerol with an acetyl-group rather than a long-chain fatty acid at the *sn*-3 position. Acetyl-TAG is similar in structure to the emulsifier ACETEM used in foods, and to plasticizers used for PVC food packaging. In order to produce large quantities of this oil, we isolated a diacylglycerol acetyltransferase gene (*Ea*DAcT) from Burning Bush and used it to engineer conventional and high-oleic lines of Camelina. Oil compositions with up to 85 mol% acetyl-TAG were achieved in lines expressing *Ea*DAcT together with suppression of Camelina competing diacylglycerol acyl transferases. There were minor or no effects on seed yield, seed weight, or harvest index of the field-grown transgenic plants. Acetyl-TAG produced in Camelina could be separated from conventional TAG by molecular distillation or purified by column chromatography. The purified acetyl-TAG crystallization temperature (by DSC cooling) was reduced by 30 C compared to control TAG; Viscosity was reduced by 27%; and caloric content was reduced by 6%. Cloud point and pour point of di-oleoyl-acetyl-TAG were substantially reduced compared to triolein. Two acres (1 ha) grown in Canada in 2015 yielded 2400 lbs (1100 kg) of seeds for further oil extraction and testing

Metabolic Engineering of Arabidopsis and Temperate Oilseed Crops to Produce Punicic Acid. E. Mietkiewska[1], R. Miles[1], A. Wickramarathna[1], A. Scheick[2], S. Shah[1], Z. Song[1], A.F. Sahibollah[1], M.S. Greer[1], C.J. Field[1], and R.J. Weselake*[1], [1]Dept. of Agricultural, Food, & Nutritional Science, University of Alberta, Canada, [2]Bioresources Technologies, Alberta Innovates Technology Futures, Canada.

Punicic acid (18:3$\Delta^{9cis,11trans,13cis}$) is a conjugated fatty acid which accumulates to high levels in the seed oil of the sub-tropical fruit pomegranate (*Punica granatum*). Recent experiments demonstrated that 10 µM punicic acid is effective in substantially inhibiting the growth of human breast cancer cells. Punicic-acid enriched oils may also be useful as industrial drying agents due to their rapid oxidation. Production of punicic acid-enriched oils in temperate oilseed crops could increase the global supply of this type of value-added oil. cDNAs encoding enzymes involved in punicic acid production in pomegranate were used to transform the *Arabidopsis thaliana fad3(fatty acid elongase)fae1* mutant, canola-type *Brassica napus* DH12075 and low α-linolenic acid (18:3$\Delta^{9cis,12cis,15cis}$) Linola-type flax (*Linum usitatissimum*) in a seed-specific fashion. About 22% (w/w) punicic acid was produced in the oil of Arabidopsis T_3 seeds co-expressing pomegranate *FATTY ACID DESATURASE* (*FAD2*) and *FADX.* Canola and Linola could also be engineered to produce punicic acid. The linin promoter was more effective than the napin promoter in enhancing punicic acid accumulation in Arabidopsis. Introduction of pomegranate diacylglycerol acyltransferase 2 resulted in further increases in puncicic acid content in Arabidopsis and Linola.

Maximizing TAG Accumulation in Plant Biomass by Combinatorial Metabolic Engineering. J.R. Petrie, T. Vanhercke, A.G. Green, and S.P. Singh, CSIRO, Australia.

Supply of vegetable oils as a major commodity faces continuous pressure. Global demand is expected to double in the next two decades due to increasing world population and rising petroleum prices. Increasing limitations on arable land and agricultural inputs mean it will be difficult to meet this additional demand with current oilseed-based production systems. The concept of producing oil in the leaves and stems of high biomass species has attracted attention as a way to intensify oil production.

We here report the accumulation of over 32% triacylglycerol (TAG, dry weight) in leaf tissue of *Nicotiana* species. This was achieved by combinatorial metabolic engineering in which we increased fatty acid biosynthesis ('Push') by limited overexpression of WRI1, increased TAG assembly ('Pull') by expressing DGAT1, and encouraged oil body formation ('Packaging') by expressing oleosin in plant leaves. This engineering resulted in the accumulation of 15% TAG in tobacco leaves. To further increase TAG levels we then interrupted a futile TAG synthesis/breakdown cycle by silencing SDP1, a TAG lipase. In a separate event we also overexpressed the LEC2 transcription factor using a

senescence promoter. Both approaches resulted in additional TAG accumulation exceeding 30% on a dry weight basis. Acknowledgements: James Petrie was awarded a PhD from the Australian National University and now works at Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) on plant metabolic engineering. James also leads a cluster of projects focused on increasing the yield of plant-derived oil by producing oil in biomass (leaves and stems) rather than seeds only. This work will help meet the demand created by growing populations and oil consumption. James has also been instrumental in modifying plant seeds to produce long-chain omega-3 oils. These have strong health benefits for consumers and these plants will both reduce pressure on fish stocks as well as create a new land-based production platform.

Sustainable Improvement of Oil Palm Through Biotechnology. G.K.A. Parveez, R. Singh, M.O. Abdullah, E.T.L. Low, O.A. Rasid, M.A.A. Manaf, and R. Sambanthamurthi, Malaysian Palm Oil Board, Malaysia.

Oil palm is the most productive vegetable oil crop that can be used either as edible foodstuff or industrial feedstock. In order for the oil palm industry to remain competitive in the long term, diversification of research into profitable areas, such as palm biotechnology is a necessity. Biotechnology offers tremendous opportunities for further improvement of oil palm yields. The idea is to deploy appropriate biotechnology tools to obtain highest yielding trees, with good quality oil and minimal environmental footprint. In this regard, MPOB made a major breakthrough in sequencing the genome of oil palm, which allowed the identification of genes influencing important agronomic traits. Conversion of these discoveries into molecular diagnostic assays will also help improve breeding efficiency. Apart from conventional breeding, MPOB also has an active tissue culture programme to clone the highest yielding palms. Uniformity of planting materials via cloning, offers the opportunity to increase yields up to 30%. However, abnormalities observed in a small percentage of the clones, has restricted large scale commercialization. Nonetheless, it has opened up opportunities for deciphering the epigenome of oil palm to understand molecular basis of clonal abnormality. The advancement of genome research also helps to expedite the transgenic research. Recent development in oil palm genome efforts and related studies will be elaborated.

BIO 4.1/S&D 4: Biosurfactants and Biodetergents

This session developed in conjunction with the Surfactants and Detergents Division.
Chairs: D.K.Y. Solaiman, USDA, ARS, ERRC, USA; D.G. Hayes, University of Tennessee, USA; and H.E. Byrne, Huntsman Performance Products, USA

A Survey of Biosurfactant Rhamnolipid Production and Applications. D.K.Y. Solaiman and R.D. Ashby, USDA, ARS, ERRC, USA.

Rhamnolipids (RLs) are microbial-produced glycolipids in pilot-scale production for applications in niche markets. Presently, RL is mainly used as biobased surfactants in cleaning-product formulation. However, other properties of RL could present new opportunities for their commercial applications. In this review presentation, various production systems for RLs will be surveyed to evaluate the volumetric productivity potentials of the processes. The antimicrobial and other biological activities of RLs will also be reviewed to provide data-set useful for product developments.

Rhamnolipid Composition, Modification, and Soil Adsorption. S. Miao[1,3], S. Soltani Dashtbozorg[1,2], A. Sancheti[1], K. Invally[1], and L.K. Ju*[1], [1]University of Akron, USA, [2]Chromatan Corp., USA, [3]George Washington University, USA.

Rhamnolipids are well known microbial surfactants. We recently investigated the potential effects of cultivation factors, mainly substrate and cultivation time, on the congener composition of rhamnolipids produced along anaerobic *Pseudomonas aeruginosa* fermentation. We also converted rhamnolipids to several derived compounds, ranging from nonionic surfactants to polymers. For applications as agricultural biopesticide or bioremediation, we further characterized the soil adsorption behaviors of rhamnolipids. Results of these studies will be presented.

A New Synthetic Platform to Create Known and Novel Bioinspired Glycolipids. C.J. Boxley[1], J.E. Pemberton[2], and R.M. Maier[2], [1]GlycoSurf, LLC, USA, [2]University of Arizona, USA.

Since their initial discovery and introduction to the marketplace in the 1960's, microbially produced glycolipids (biosurfactants) have experienced a steady rise in interest and potential applications as "green" alternatives to traditional petroleum-based specialty surfactants. This is due to a combination of their environmentally friendly characteristics, including low toxicity and ready biodegradability, as well as their excellent surfactant attributes. A new technology recenlty introduced builds on these advantages by providing a platform to synthesize a wide variety of glycolipids. This presentation will discuss some of the advantages associated with using synthetic approaches to produce glycolipids including: 1) the ability to chemically synthesize single congeners rather than undefined and unreproducible congener mixtures as produced by biosynthesis, 2) the ability to tailor both the head and tail groups to produce surfactants with a wide range of properties that can be used for specific applications, and 3) the potential to use waste stock materials to produce these surfactants cheaply.

Novel Sophorolipid-based Biosurfactants by Metabolic Engineering: Production and Application. I.N.A. Van Bogaert[1], S.L.K.W. Roelants[2], and W. Soetaert[1,2], [1]Ghent University, Belgium, [2]Bio Base Europe Pilot Plant, Belgium.

Through metabolic engineering of the unconventional yeast *Starmerella bombicola* we created several new-to-nature and tailor-made biosurfactants. Indeed, despite the clear advantages of biosurfactants, their overall use is hampered by the lack of structural variation. This is in sharp contrast to chemically produced surfactants where one can introduce variation by simply changing the building blocks. Structural variation is essential as (bio)surfactants find application in a very broad range of sectors. We alleviated this fundamental limitation by developing a generic biotechnological production technology for glycolipid biosurfactants, based on the very efficient biosurfactant producing yeast *S. bombicola*. By metabolic engineering, most structural parts of the glycolipid biosurfactant molecule can be controlled: fatty acid tail, sugar moiety, acetylation and lactonization. For each target molecule, an industrial applicable fermentation and downstream process was developed and the molecules are evaluated for various applications such as cleaning, cosmetics, medics and nanoscience. Our approach thus covers the whole innovation chain from basic research to production and application development. To achieve this goal, a complementary consortium of academic and industrial partners was formed that covers the whole range of required expertise.

Sophorolipid Modifications: Advantages of a New Pathway. D.W.G. Develter, Ecover Coordination Centre, Belgium.

As sophorolipids are finding their way to the detergent and cosmetic market, the search for higher foaming sophorolipids continues. A state of the art overview of chemical and biotechnological modifications is given which shows that none of the commercially viable modifications outperforms wild type sophorolipids. Finally the synthesis of a modified sophorolipid species is described, the physicochemical behaviour of which for the first time ever exceeds that of wild type sophorolipids.

Surfactants Based on Algae Oil. G.A. Smith, Huntsman Performance Products, USA.

Modern day surfactants are based on natural, petrochemical or a combination of natural and petrochemical feedstocks. With the recent emphasis on sustainability,

surfactants based on natural feedstocks are of considerable interest. Palm-based natural alcohols are produced in regions where deforestation is a concern, have long supply chains to developed markets and is a food source for human beings.

An alternative feedstock which overcomes the deforestation concerns and is not used for food is algae. There are thousands of different algae species which can grow in fresh or salt water. Work was performed to optimize the growth conditions for different types of freshwater algae in laboratory photobioreactors (PBR). Light frequencies and fertilizer concentrations were varied to achieve the optimum growth conditions.

The oil extracted from algae was used to make nonionic surfactants by low temperature transesterification with an ethoxylated polyol. The process is fast and produces only trace amounts of dioxane and residual EO. The products are light colored, low viscosity liquids with no gel phase upon dilution in water. Surface properties and detergency looks similar to conventional natural alcohol ethoxylates. Work was performed to optimize the molecular structure for different applications including detergency, rheology modification and foam control for home and personal care products and polyurethane foam applications.

Next Generation Castor Oil Ethoxylates. H.E. Byrne, G.A. Smith, M.T. Meredith, and C. Cleary, Huntsman Performance Products, 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.

In this work, an investigation was carried out in order to see what benefits would be shown with the new COE technology compared to the direct ethoxylation products. Properties compared include EO or 1,4-dioxane content, cloud points and foam profiles among many others. Comparisons with industry specific formulations will also be reported.

New and Emerging Biobased Surfactants: A Review. D.G. Hayes, Dept. of Biosystems Engineering & Soil Science, University of Tennessee, USA.

This presentation will review three new biobased surfactants described in recent issues of the Journal of Surfactants and Detergents (JSD). First, in a series of two papers, Cai and coworkers have developed a nonionic surfactant formed from low molecular weight chitosan and an epichlorohydrin-derivatized form of dehydroabietic acid, a derivative of pine tree rosin (JSD 17:493 [2014] and 18: 463 [2015]). The derivatives lowered the surface tension of water to 36 mN/m and were effective emulsifiers. Second, Negm and coworkers have produced biobased surfactants formed through esterification of jatropha oil fatty acid ethoxylates and p-aminobenzoic acid, the latter derived from vanillin (JSD 18:1011 [2015]). The derived surfactants were effective as anticorrosion inhibitors on carbon steel. Third, Heidelberg and co-workers produced sugar-based surfactants that contained an amide linkage for use as an oil-based emulsifier through converting the primary OH group of methyl glucoside into an azide group, and then forming an amide linkage between the azide group and a fatty acyl derivative through the Staudinger coupling reaction (JSD 17:1141 [2015]). The surfactants possessed a high Krafft point temperature and tended to form hexagonal phases in solution; but, long-chain derivatives were capable of emulsifying water into oil.

BIO 4.2/IOP 4: Biofuels II

This session developed in conjunction with the Industrial Oil Products Division.
Chairs: T. Aki, Hiroshima University, Japan; and R.M. Burton, Novozymes, USA

Influence of Corn Oil Recovery on Life-cycle Greenhouse Gas Emissions of Corn Ethanol and Corn Oil Biodiesel. Z. Wang[1], J.B. Dunn[2], J. Han[2], and M.Q. Wang[2], [1]EcoEngineers LLC, USA, [2]Systems Assessment Group, Energy System Div., Argonne National Lab., USA.

Corn oil recovery and conversion to biodiesel has been widely adopted at corn ethanol plants recently. The US EPA has projected 2.6 billion liters of biodiesel will be produced from corn oil in 2022. Corn oil biodiesel may qualify for federal renewable identification number (RIN) credits under the Renewable Fuel Standard, as well as for low greenhouse gas (GHG) emission intensity credits under California's Low Carbon Fuel Standard (LCFS). Because multiple products [ethanol, biodiesel, and distiller's grain with solubles (DGS)] are produced from one feedstock (corn), however, a careful co-product treatment approach is required to accurately estimate GHG intensities of both ethanol and corn oil biodiesel and to avoid double counting of benefits associated with corn oil biodiesel production.

This study develops four co-product treatment methods: (1) displacement, (2) marginal, (3) hybrid allocation, and (4) process-level energy allocation. Life-cycle GHG emissions for corn oil biodiesel were more sensitive to the choice of co-product allocation method because significantly less corn oil biodiesel is produced than corn ethanol at a dry mill. Corn ethanol life-cycle GHG emissions with the displacement, marginal, and hybrid allocation approaches are similar (61, 62, and 59g CO2e/MJ, respectively). With the process-level allocation approach, ethanol's life-cycle GHG emissions are lower at 46g CO2e/MJ. Corn oil biodiesel life-cycle GHG emissions from the marginal, hybrid allocation, and process-level energy allocation methods were 14, 59, and 45g CO2e/MJ, respectively. Sensitivity analyses were conducted to investigate the influence corn oil yield, soy biodiesel, and defatted DGS displacement credits, and energy consumption for corn oil production and corn oil biodiesel production. This study's results demonstrate that co-product treatment methodology strongly influences corn oil biodiesel life-cycle GHG emissions and can affect how this fuel is treated under the Renewable Fuel Standard and Low Carbon Fuel Standard.

Novel Alpha-amylase Technology for Enhanced Fermentation Efficiency and Corn Oil Recovery in Industrial Biofuel Production. P. Mandulak, R.M. Burton*, J. Matthews, and K. Bertz, Novozymes North America, USA.

In commercial dry grind corn ethanol production, over 75% of ethanol production plants employ corn oil recovery systems. Corn oil extraction has become an essential income stream which yields a valuable corn oil by-product for animal feed or biodiesel production. Corn used for industrial ethanol production contains approximately four percent fat content by weight. Extracting the full percentage of these lipid materials is a challenge for production facilities and requires surfactant chemistries to enhance mechanical extraction. A new approach to significantly increase oil extraction rates is the utilization of novel alpha-amylases enzyme package developed to enhance the oil yields in biofuel plants. This research will review commercial use of these enzymes in dry-grind corn processing including how these combinations can reduce chemical inputs of nitrogen in feedstreams, increase production yield, and enhance the volume of corn oil extraction.

Maximizing Oil Recovery from Corn-soy Slurry After Fermentation with *Saccharomyces cerevisiae*. J.K. Sekhon[1,2], K.A. Rosentrater[2,3], and S. Jung[4], [1]Dept. of Food Science & Human Nutrition, Iowa State University, USA, [2]Center for Crops Utilization Research, USA, [3]Dept. of Agricultural & Biosystems Engineering, Iowa State University, USA, [4]Dept. of Food Science & Nutrition, California Polytechnic State University, USA.

Utilizing co-products of enzyme assisted aqueous extraction process of soybeans in corn fermentation has shown promising results with significant increase in ethanol yield (20%) and decrease in fermentation time (44 h) compared to corn only fermentation. However, to maximize economic returns oil can be recovered from the slurry after fermentation. Removal of oil from the fermented slurry also leads to production of high quality DDGS (dried distillers grains with solubles) used for animal feed. Efficient sustainable methods are required to maximize oil recovery. In the present study, soy skim (protein rich) and insoluble fiber (IF; carbohydrate rich), co-products of EAEP, were utilized in dry-grind corn fermentation. The effect of 1) fiber hydrolyzing enzymes, 2) proteases, and 3) surfactants alone or in combination on oil recovery from [corn only], [corn + skim], [corn + insoluble fiber], [corn + (skim + insoluble fiber)] slurries were investigated. The process was optimized for type, concentration, and point of addition of enzymes and surfactants. Maximum oil recovery was achieved from [corn only] and [corn + insoluble fiber] slurries when pectinase, cellulase and Fermgen protease were added to corn-soy slurry during fermentation and when Tween80 was used as a surfactant or demulsifier.

Keynote Presentation: Fuels from Oils and Fats: Recent Developments and Perspectives. M. Mittelbach, Inst. of Chemistry, University of Graz, Austria.

The future of fuels made out of fats and oil is evaluated and discussed. Fats and oils today represent the main source for alternative transport fuels, especially for FAME (biodiesel) and HVO (hydrotreated vegetable oils). Recent developments in FAME and HVO production and utilization are highlighted, including microbial oils as potential feedstocks. The use of new heterogeneous catalysts as well as promising reactor designs for FAME production is discussed as well as bio-catalytic approaches. Since HVO production technology is based mainly on current mineral oil industry processing methods, the scientific literature is not as extensive as that of FAME production. The main reaction routes of HVO are a combination of hydrogenation, decarboxylation, decarbonylation, hydroisomerization and cracking under high pressure and temperatures, using supported and unsupported heterogeneous metal catalysts. Own experiments using non food feedstocks as starting material like waste animal fat or tall oil under heterogeneous catalysis are presented. The hype concerning the use of algae based material has been cooled down when comparing different production routes for cultivation at different locations including the production costs. Industrial significant progress has not occurred so far, leading to some disillusionment with this approach. Therefore, a clear strategy should be developed for upstream and downstream processes with parallel evaluation of life cycle assessment (LCA). In addition, energy intensive steps of biomass drying and solvent extraction should be avoided by using intelligent technologies, such as in situ processing of wet biomass.

Production of Biomethane and Functional Lipids from Marine Macroalgae. T. Aki[1,2], K.H.V. Arafiles[1,2], K. Watanabe[1,2], Y. Okamura[1,2], T. Tajima[1,2], Y. Nakashimada[1,2], and Y. Matsumura[1,2], [1]Hiroshima University, Japan, [2]JST-CREST, Japan.

Marine biomass has attracted great attention in the fields of renewable bioenergy and value-added industrial and biological materials production due to the promised sustainability of its use. We have succeeded at using macroalgae (1) to produce biomethane using marine sediment-derived culture, and (2) to produce functional lipids such as polyunsaturated fatty acids (PUFA), xanthophylls, and terpenoids by using the marine protists, the thraustochytrids. Biomethane was produced through a fed-batch methane fermentation method that used a marine sediment-derived microbial consortium acclimated to high salinity. The consortium was able to the degrade algal polysaccharides and assimilate the resulting organic acids to produce biomethane. In addition to this, a number of bacteria and fungi that can degrade and convert algal saccharides into suitable substrates for thraustochytrid were obtained to serve as intermediate biocatalysts for a composite culture system that ultimately produces PUFA, xanthophylls and terpenoids. Hydrothermal treatment of algal mass and recovery of residual metals for complete utilization of marine macroalgae were also introduced.

Impact of Biochemical Composition on Susceptibility of Algal Biomass to Acid-catalyzed Pretreatment for Sugar and Lipid Recovery. T. Dong, N.J. Nagle, P.T. Pienkos, and L.M.L. Laurens*, National Renewable Energy Lab., USA.

One of the major challenges associated with algal biofuels production in a biorefinery-type setting is improving biomass utilization in its entirety, increasing the process energetic yields and providing economically viable and scalable co-product concepts. Here we focus on the impact of compositional characteristics of biomass on the susceptibility to pretreatment in a biorefinery-type setting. The release of monomeric carbohydrates in the aqueous phase and extractability of the lipid fraction was measured based a response surface methodology, allowing for the analysis of interaction effects for three algal strains; C. vulgaris and S. acutus and N. granulata representing three different nutritional metabolic phases. We identified distinct patterns of acid and temperature combinations on the yield of released monomeric carbohydrates and lipid extractability. Four cultivation conditions of high potential value for a combined sugar- and lipid-based biofuels process were identified, and are based on experimental results obtained from pretreatment and lipid extraction. The results indicate that acid pretreatment has potential to be applicable for a vast range of biomass samples to obtain high energy yields, but that the exact conditions and optima are dependent on the strain and likely the starting composition of the biomass.

Confocal Raman Microscopy for Quantitative Analysis of Microalgal Lipid Contents: An Integrative Workflow for Rapid *in situ* Analysis. S.K. Sharma[1], D.R. Nelson[2], R. Abdrabu[2], B. Khraiwesh[2], K. Jijakli[2], M.J. O'Connor[3], T. Bahmani[2], H. Cai[2], S. Khapli*[1], R. Jagannathan[1], and K. Salehi-Ashtiani[2], [1]Div. of Engineering, New York University Abu Dhabi, United Arab Emirates, [2]Lab. of Algal, Systems, & Synthetic Biology, Div. of Science & Math, Center for Genomics & Systems Biology (CGSB), New York University Abu Dhabi, United Arab Emirates, [3]Core Technology Platform, New York University Abu Dhabi, United Arab Emirates.

Algal biofuels have received worldwide attention as sustainable resources to substitute fossil fuels. The economical feasibility of industrial production of algal

biofuels with desired molecular characteristics is likely to require the discovery and isolation of new algal species and subsequent strain optimization. There is a need to develop analytical techniques for rapid screening of cells for the desired lipid molecular characteristics. Here we describe an integrated workflow for *in situ* analysis of algal lipids through Raman microscopy, a technique that enables *in vivo* monitoring of lipid contents in a rapid, quantitative, and label-free, manner. To establish the workflow, we refined existing Raman analysis techniques to obtain better discrimination in chain-length and saturation of lipids. We performed characterization of lipid content of novel aquatic and soil microalgal isolates as well as lipid-expressing cells obtained in a mutagenesis screen. The workflow enabled quantitative analysis of the different saturation states among the isolates. Cell-to cell variations among mutagenized *C.reinhardtii* cells grown under identical conditions were also observed. Our results validate the utility of Raman analysis in determination of the key parameters for the selection and engineering of microalgae necessary for optimal production of biofuels.

Integration of Experimental Systems with Engineering Process Modeling for Sustainability Assessment of Microalgal Biofuel Systems. J.C. Quinn, Mechanical & Aerospace Engineering, Utah State University, USA.

Globally, we face an energy crisis due to an increase in energy consumption combined with the negative effects associated with traditional fossil energy sources. A variety of green technologies coming together to address environmental concerns, while meeting global increases in energy demand, is likely to be a critical component of the solution. This seminar presents the integration of experimental systems with engineering process modeling for sustainability assessment applied to microalgal biofuel systems. Sustainability modeling includes techno-economic assessments, life cycle assessments, and scalability assessment through resource availability. Data feedback from sustainability modeling is used to highlight areas for focused research and development on the metrics of economic viability and environmental impact. Further, engineering process modeling is used to identify knowledge gaps for experimental work. The integration of sustainability modeling with experimental systems is a valuable tool that can decrease experimental design space and focus research in areas that can accelerate commercialization. Experimental research spans the value chain of a microalgal biorefinery system with the focus on the generation of data for system model validation. System models are leveraged for sustainability assessments with data feedback to experimental work for focused research and development. Results show microalgae systems have the potential to be economically competitive with traditional fuels while providing a valuable environmental service.

BIO 5: General Biotechnology

Chairs: J. Ogawa, Kyoto University, Japan; and T.A. McKeon, USDA, ARS, WRRC, USA

Lipases/Acyltransferases, Promising Biocatalysis for Simple and Efficient Valorization of Biomass Lipids in Biorefinery Processes. M. Subileau[1,2], A.H. Jan[2], V. Perrier[1,2], and É. Dubreucq[1,2], [1]Montpellier SupAgro, France, [2]UMR IATE, France.

The valorization of lipids through biorefinery processes often requires energy and time consuming procedures, such as solvent extraction steps and pre-drying of the biomass. Our experience in the identification and optimization of enzymes has allowed us to build a collection of peculiar lipases that exhibit exceptional ability to catalyze acyltransfer reactions in high water content media. These wild-type, mutant and chimeric enzymes are related to the lipase/acyltransferase of *Candida parapsilosis* (CpLIP2) and to the lipase A of *C. antarctica* (CaLA). Several rounds of selection and enzyme engineering have allowed the production of remarkable enzymes. In simple oil-buffer-alcohol media, these biocatalysts have shown ability to synthetize fatty acid esters at high yield and productivity compared to classical lipases. Even at very low alcohol concentration (a_w>0.95), they catalyze direct alcoholysis of the acyl-donor through kinetically controlled reaction, allowing the synthesis of fatty acid esters beyond the thermodynamic equilibrium. Considering the large range of substrate affinities of these enzymes and their high specific activity, their implementation in biorefinery processes could allow simple and eco-efficient valorization of the biomass lipid fraction, without the need to first concentrate or dry neither the biomass nor the alcohol.

New Tale from an Old Story: Why and How Can Candida Antartica Lipase A Produce Omega-3 PUFAs Concentrates in High Selectivity for Industrial Interest? Y. He[1,2], B. Chen[2], S. Kodali[1,3], J. Li[1], and Z. Guo*[1], [1]Aarhus University, Denmark, [2]Fujian Normal University, China, [3]University of Borås, Sweden.

The healthy benefit from ω-3 PUFAs has been widely acknowledged; however, The quality and quantity of ω-3 PUFAs in marine resource have been declined dramatically; therefore production of high quality ω-3 PUFAs concentrates in an environment-friendly manner is a challenge for biotechnology scientists.

This work will present our recent exciting finding in developing the process to concentrate ω-3 PUFAs via an enzymatic approach. In a non-aqueous system, suprisingly non-spefific CAL-A is capable to directly concentrate ω-3 PUFAs from 20-30% to up to >90%. CAL-A demonstrated such highly nonpreferability towards Stearidonic acid, Eicosapentaenoic acid and Docosahexaenoic acid. The products to confirm the effectiveness of the process have verified by regio-distribution fatty acid composition analysis, GC, TLC-FID, and decoupling 13CNMR analyses. This approach is generally applicable for concentration of fish oils and microalgae oils in high selectivity and high efficiency. In combination with short path distillation process, this novel approach demonstrated its industrial relevance and application potential in food and drug industries.

Improving Lipases/Acyltransferases to Face the Requirements of Biorefinery Processes by Rational Design. A.H. Jan[2], M. Subileau[1,2], C. Deyrieux[1], V. Perrier[1,2], and É. Dubreucq[1,2], [1]Montpellier SupAgro, France, [2]UMR IATE, France.

Transesterification in aqueous media is becoming a priority challenge in lipid biotechnology in order to develop more eco-friendly and efficient biocatalytic processes in systems containing both polar and hydrophobic substrates. In this context, our group has explored for numerous years the high potential of the lipase/acyltransferase CpLIP2 from *Candida parapsilosis* and of several of its homologs, that catalyze efficiently acyltransfer reactions in lipid/water media with high a_W (>0.9). Heterologous expression of CpLIP2 homologs allowed us to functionnally characterise several new lipases/acyltransferases, among which various levels of acyltransfer activity in aqueous medium where observed. The comparison between the elucidated crystallographic structure of CAL-A from *Pseudozyma antarctica* [(1)], that has a low acyltransfer activity at high a_W, and homology 3D models of CpLIP2 [(2)], of some of its relevant site-directed mutants and of the other new lipases/acyltransferases, allowed the identification of putative key amino-acid residues involved in acyltransfer *vs* hydrolysis activity. Site-directed mutants were then designed to verify the role of these residues and to develop improved biocatalysts, including an improved CAL-A mutant.

[(1)] Ericsson et al. (2008) *J Mol Biol.* 376:109-19.

[(2)] Subileau et al. (2015) *BBA-Prot Proteom*, 1854:1400-1411.

Palm Stearin/Shea Stearin-based Cocoa Butter Equivalents. B.H. Kim[1] and I.H. Kim[2], [1]Sookmyung Women's University, Republic of Korea, [2]Korea University, Republic of Korea.

The aims of this study were to produce a fractionated palm stearin enriched in 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), and to prepare cocoa butter equivalents (CBEs) by blending the fractionated palm stearin with shea stearin, 1,3-distearoyl-2-oleoyl-glycerol (SOS)-rich fats. A liquid fraction was produced from palm stearin and it contained ~37.3w/w% POP, ~6.4w/w% 1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol (POS), and ~0.8w/w% SOS. The liquid fraction was further crystallized to obtain a solid fraction with ~53.0w/w% POP, ~9.1w/w% POS, and ~1.1w/w% SOS. The CBEs were prepared by blending the fractionated palm stearin and shea stearin in a weight ratio of 40:60. The CBEs were blended with cocoa

butter in weight ratios (CBEs:cocoa butter) of 5:95–90:10. The 5:95, 10:90, 20:80, and 30:70 blends showed similar melting/crystallization temperature ranges and enthalpies to those of cocoa butter. They also showed similar changes in solid fat content to those of cocoa butter as a function of temperature. These results collectively suggest that the CBEs can be blended with cocoa butter at 30w/w% for the manufacture of chocolate products without significantly altering their physical properties.

Production of Pinolenic Acid Rich Triacylglycerol via Direct Esterification Using Mixed Lipase. T. Kim[1,2], H. Kim[1,2], G. Chijioke[1,3], and I.H. Kim[1,2], [1]Dept. of Food & Nutrition, Korea University, Republic of Korea, [2]Dept. of Health Science, Graduate School, Korea University, Republic of Korea, [3]Dept. of Integrated Biomedical & Life Science, Korea University, Republic of Korea.

Pinolenic acid (PLA), all-cis-5,9,12-18;3 is a polyunsaturated fatty acid of pine nut oil and it has several physiological dietary effects. PLA-enriched triacylglycerol (TAG) was successfully synthesized by lipase-catalyzed esterification using mixed lipase. The mixed lipase employed composed of Novozym 435 from *Candida Antarctica* and Lipozyme TM IM from *Thermomyces lanunginosus*. For the 1st step, Novozym 435-catalyzed esterification of PLA-enriched fatty acid and glycerol was carried out for the synthesis of TAG for 2 h. For the 2nd step, Lipozyme TL IM was added into the reaction mixture from 1st step and the remaining reaction was performed for 22 h. The optimum conditions of the 1st step were a temperature of 60 °C an enzyme loading of 2%, and a vaccum of 0.67 kPa, respectively. The optimum reaction conditions of the 2nd step were a temperature of 60 °C an enzyme loading of 13%, and vacuum of 0.67 kPa, respectively. Finally, the maximum TAG yield of ca. 94.3% was obtained via two-step lipase-catalyzed esterification.

Production of a Bioactive Lipid-based Delivery System from Ratfish Liver Oil by Enzymatic Glycerolysis. P. Arranz-Martínez[1], M. Corzo-Martínez*[1], L. Vázquez[1], N. Menéndez[1], G. Reglero[1,2], and C.F. Torres[1], [1]Inst. of Food Science Research, CIAL (CSIC-UAM), Spain, [2]Imdea-Food Inst., CEI (CSIC-UAM), Spain.

Often bioactive ingredients with promising effects in assays *in vitro* do not provide similar efficacy when they are tested *in vivo* mainly because of their low bioavailability. To improve this aspect, bioactive compounds are frequently formulated in association to lipids, known as lipid-based delivery systems (LDS). In this sense, utilization of alkylglycerols as LDS could also provide beneficial effects on human health besides the vehiculization of bioactive ingredients.

The aim of the present work was the design of bioactive LDS with self-emulsifying properties by enzymatic glycerolysis from ratfish liver oil, a natural source rich in alkylglycerols. For process optimization, several commercial lipases were tested. The influence of adding monoolein or using GRAS solvents in the enzymatic glycerolysis was also assessed. Samples were characterized by HPLC-ELSD.

A potential self-emulsifying system comprised of 72% (w/w) of mono-, di-acylglycerols, and monoesterified alkylglycerols was obtained when the process was carried out at 40°C, in the presence of *Candida antarctica* lipase and 67% (w/w) of cyclopentanone. At these conditions, the process was scaled-up to pilot plant. Similar results to that observed at laboratory scale were attained. Therefore, enzymatic glycerolysis can be an efficient and scalable method to obtain LDS, useful in the formulation of functional ingredients.

Cell Factory Engineering of Oleaginous Yeast *Yarrowia lipolytica* for Production of Renewable Oleochemicals and Isoprenoids. X.C. Xiong and S.L. Chen, Biological Systems Engineering, Washington State University, USA.

Microbial lipid has been explored as an alternative resource for production of fungible biofuels, but there are significant economic and commercial constraints. To address this challenge, we have developed a biotechnology platform for producing both lipid-based compounds and isoprenoids as high-value co-products from renewable feedstock by metabolic engineering of the oleaginous yeast *Yarrowia lipolytica*. We have genetically modified *Y. lipolytica* for production of fatty alcohol, the key oleochemical with huge market. We extended this pathway to produce the tailor-made long-chain wax esters with wide applications for making lubricants and cosmetic products. Lipid body formed in *Y. lipolytica* has been designed and engineered as a compartment for storage of isoprenoids of interests. It can not only increase the sink capacity of the host, but also alleviate the physiological perturbations conferred by the hydrophobic products, thus increasing the yield of isoprenoids. Although we focused on a limited number of molecules served as antioxidant (squalene), carotenoid nutraceutical (lycopene) and steroid hormone (ergosterol), this platform can be extended to other isoprenoid family with necessary biosynthetic pathways. Our technology fills a critical technical gap by accelerating economy, prosperity and environmental sustainability of oil industry.

Effect of Supercritical CO_2 and Type of Co-solvent for Extraction of Lipids and Terpenics from Guayule Biomass (*Parthenium argentatum*). T. Punvichai[1,2], E. Tardan[1], S. Palu[1], and D. Pioch[1], [1]UR BioWooEB - Biorefinery Team, CIRAD, France, [2]Prince of Songkla University, Surat Thani Campus, Thailand.

Guayule (*Parthenium argentatum*), a perennial crop under semi-arid climate, producing polyisoprene (Guayule rubber, GR) and resin, is the most probable alternative source of natural rubber. To date efforts aimed at marketing GR, while the bagasse (GB) (90%-dw, dry weight) left after water-based extraction remains underused. Thus Cirad is investigating a "green" process for extracting valuable compounds from GB, including lipids. After having obtained preliminary results, showing that a co-solvent is necessary in addition to SC-CO_2, we now investigate the influence of operating parameters. Under SC-CO2, resin extract is higher with ethanol as co-solvent (12.1%-dw) compared to acetone (7.8%-dw), at temperature 35°C and 300 bar, co-solvent flow rate 3mL/min, CO_2 flow rate 34.4 g/min. Acetone-based extraction under pressurized conditions above boiling point (ASE-acetone) is used as reference method. The selectivity for aromatic carboxylic acids (cinnamic, *p*-anisic) initially linked to sesquiterpenes, is better with SC-CO_2-ethanol compared to SC-CO_2-acetone. The minor components are lipids rich in C18:2, C16:0, C18:3, in between cotton and soybean oils, although poorer in C18:1. The unsaponifiable contains sesquiterpenes, whose extraction is mainly dependent on pressure but not temperature with SC-CO_2-ethanol.

BIO-P: Biotechnology Poster Session

Chairs: B.H. Kim, Chung-Ang University, Republic of Korea; and S. Kishino, Kyoto University, Japan

2. **Antiobesity and Hypolipidemic Effects of Dietary Structured Pinolenic Triacylglycerols in Diet-induced Obese Mice.** M.Y. Chung[1], H. Woo[2], H.D. Choi[1], I.W. Choi[1], and B.H. Kim[3], [1]Korea Food Research Inst., Republic of Korea, [2]Chung-Ang University, Republic of Korea, [3]Sookmyung Women's University, Republic of Korea.

This study aimed to examine the antiobesity and hypolipidemic effects of different levels 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, respectively, 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 increase in body weight gain, which was significantly decreased by 5% dietary supplementation with SPT13 or SPT44. HFD-fed mice showed significantly higher epididymal and retroperitoneal fat and liver weights than normal diet (ND) controls. SPT44 at 5% reduced epididymal and retroperitoneal fat weights significantly compared to HFD, whereas SPT13 at 5% did not. There was no difference in liver weight between groups ND and SPT44 at 5%. Both SPT13 and SPT44 at 5% significantly reduced serum triglyceride despite no significant regulation in total cholesterol level. LDL cholesterol level was significantly attenuated by SPT44 at 5% only that is otherwise increased by HFD. Thus, these results suggest that 5% dietary supplementation with SPT44 has antiobesity and hypolipidemic activities in diet-induced obese mice.

3. **Lipase-catalyzed Synthesis of a Novel Xylitol Ester of 7,10-dihydroxy-8(*E*)-octadecenoic Acid.** H.G. Lee[1], Q. Long[1], C.T. Hou[2], and H.R. Kim[1], [1]School of Food Science & Biotrechnology, Kyungpook National University, Republic of Korea, [2]USDA, ARS, NCAUR, Renewable Product Technology Research Unit, USA.

Hydroxy fatty acids have been widely studied because of their special properties such as antimicrobial activity, high viscosity and reactivity, compared to normal fatty acids. Among the hydroxy fatty acids, 7,10-dihydroxy-8(*E*)-octadecenoic acid(DOD) was well studied in terms of production, optimization and characterization. DOD was characterized to have strong antibacterial activities against food-borne pathogenic bacteria and plant pathogenic bacteria. In this study we tried to modify DOD molecules to exert enhanced biological activity and water-solubility. As a trial, we focused on enzymatic synthesis of DOD-saccharide esters. Several mono-saccharides were screened as a substrate for the lipase-catalysed esterification with DOD and DOD-xylitol ester was successfully produced by incubation at 50? with stirring at 200rpm for 24 hours in the presence of lipozyme RMIM The structure of a novel DOD-xylitol ester was verified using GC/MS, NMR, FT-IR analysis and biological activity was also determined.

4. **Synthesis of a Novel Biologically Active Amide Ester of 7,10-dihydroxy-8(*E*)-octadecanoic Acid (DOD) Using Lipase.** I.H. Choi[1], J.H. Jung[1], C.T. Hou[2], and H.R. Kim[1], [1]School of Food Science & Biotechnology, Kyungpook National University, Republic of Korea, [2]USDA, ARS, NCAUR, Renewable Product Technology Research Unit, USA.

Hydroxy fatty acids (HFA) are known to have industrial potential because of their special properties such as high viscosity and reactivity. Among the hydroxy fatty acids, 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) was successfully produced from oleic acid and lipid containing oleic acid by a bacterial strain *Pseudomonas aeruginosa* PR3. Recently we have shown that DOD presented strong antimicrobial activity against broad range of pathogenic bacteria. In this study, we tried to modify DOD molecules by lipase-catalyzed esterification with triethanolamine (TEA) to exert enhanced biological activity. Structure of a novel DOD-TEA ester was confirmed by GC-MS, NMR, FT-IR analysis. Antibacterial activity of DOD-TEA ester was determined against several food-born pathogenic bacteria. We found out that antibacterial activity of DOD-TEA ester was highly enhanced compared to those with DOD.

7. **Selective Enrichment of Conjugated Linoleic Acid Isomers from Their Mixtures Using a Combination of Urea Crystallization and Lipase-catalyzed Esterification.** J. Kim[1], I.H. Kim[2], H.D. Choi[3], I.W. Choi[3], and B.H. Kim[4], [1]Chung-Ang University, Republic of Korea, [2]Korea University, Republic of Korea, [3]Korea Food Research Institute, Republic of Korea, [4]Sookmyung Women's University, Republic of Korea.

This study aimed to selectively enrich *t*10,*c*12-conjugated linoleic acid (*t*10,*c*12-CLA) and *c*9,*t*11-CLA from commercial CLA mixtures using a combination of urea crystallization and lipase-catalyzed esterification. A free fatty acid (FFA) fraction containing 53.8% *t*10,*c*12-CLA and 39.1% *c*9,*t*11-CLA was produced from the CLA mixtures containing ~32% each of the two CLA isomers by a urea crystallization using methanol and urea-to-fatty acid (FA) weight ratio of 2.5:1. The CLA-enriched FFA fraction was esterified with dodecan-1-ol in a recirculating packed bed reactor using an immobilized lipase from *Candida rugosa* to further enrich the *t*10,*c*12-CLA and *c*9,*t*11-CLA in an FFA fraction and an FA dodecyl ester fraction, respectively, under the optimal conditions, i.e., temperature, 20°C; FA-to-dodecan-1-ol molar ratio, 1:1; water content, 2% of total substrates; residence time, 5 min; and reaction time, 24 h (for *t*10,*c*12-CLA enrichment) and 12 h (for *c*9,*t*11-CLA enrichment). After the reaction, an FFA

fraction with the maximum *t*10,*c*12-CLA content of 72.6% was obtained. Whereas, another FFA fraction with the maximum *c*9,*t*11-CLA content of 62.0% was recovered from the FA dodecyl ester fraction. The yields of *t*10,*c*12-CLA and *c*9,*t*11-CLA in the FFA fractions were 43.6% and 21.5%, respectively, based on their initial weights in the CLA mixtures.

8. **SC-CO_2 Extraction of Biodiesel in Rice Bran Synthesized by *in-situ* Transeseterification.** N.K. Choi[1,2] and I.H. Kim[1,2], [1]Dept. of Food and Nutrition, Korea University, Republic of Korea, [2]Dept. of Public Health Science, Graduate School, Korea University, Republic of Korea.

Biodiesel was extracted from rice bran using supercritical carbon dioxide. The oil in rice bran was converted to biodiesel via *in situ* transesterification using rice bran lipase and the biodiesel was extracted from rice bran using supercritical carbon dioxide. The transesterification was carried out under the optimum conditions of previous study, which were at a molar ratio of 1:2 (fatty acid to methanol), a temperature of 40°C, a water content of 12%, and a reaction time of 12 days. The extraction was carried out at an operating pressure of 11.7 -15.9MPa and an operating temperature of 35-55°C. Optimum pressure and temperature for efficient extraction of biodiesel from rice bran were 11.7Mpa and 45°C. Purity of biodiesel extracted at optimum condition was 96%.

9. **Characterization of Oleins and Stearins of Various Palm Oils from Interspecific Hybrids *Elaeis oleifera x Elaeis guinensis*.** N. Quezada[1], F. Orellana[2], C. Ulloa[1], O. Leon[2], and I. Zambrano[1], [1]La Fabril, Ecuador, [2]Energy Palma, Ecuador.

Palm oil is one of the most produced vegetable oils in the world. In recent years, interspecific hybrids (*E. Oleifera x E. Guinensis*) have gotten increased attention due to their tolerance to diseases, reduced height, adequate production yields, and oil with high levels of unsaturated triglycerides and natural antioxidants. There are no reports of the characterization of oleins and stearins obtained after fractionation of interspecific hybrid palm oils.

Five interspecific hybrid palm oils obtained from plantations located in Esmeraldas (Ecuador) were fractionated during an eight-hour process to obtain their oleins and stearins. After this process, these fractions were separated using vacuum filtration. Both fractions were weighted and the olein and stearin yield were calculated. The Iodine Value, Color, Melting Point, FAME, and Triglyceride profiles, Differential Scanning Calorimetry, Solid Fat Content, Cold Test, Carotene and Tocopherol content, and Oxidative Stability of both fractions were determined. Olein and stearin of palm oil from *E. Oleifera* were used as controls.

The olein yields were from 35 to 60%. The oleic acid content of the oleins was from 45 to 55%. Two of the five oleins showed good cold stability. All the oleins showed higher levels of carotenes and tocopherols than their stearins.

10. **Environmental Impact and Scalability of Microalgal Biofuel Production integrating Coal Fired Power Plant Flue Gas.** D. Hess, K. Napan, B. McNeil, and J.C. Quinn, Utah State University, USA.

Large scale production of microalgal based biofuels will require the integration of point source CO_2 sources. Flue gas integration from coal fired power plants fulfills this requirement while providing an environmental service by carbon sequestration. Heavy metals inherent in coal will ultimately be introduced to the culture system. Introduction of heavy metals have the potential to impact growth due to toxicity and negatively impact the quality of biofuel and other microalgal derived products. Heavy metals As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V, and Zn were added to microalgae (*Nannochloropsis salina*) growth medium at concentrations representative of 7-day growth periods using flue gas as the carbon source. Heavy metal introduction resulted in an average decrease of 52% in biomass yield and 19% in lipid content. Microalgae biomass was processed into biodiesel through one of two different *in situ* transesterification techniques (acid-catalyzed or supercritical methanol conversion). Effects of heavy metals on biofuel production were quantified. Compared to the control, total production of biofuel from the contaminated system was decreased by over 50% for both conversion types. Results are extrapolated to a national scale illustrating the impact microalgae can have on 2030 DOE renewable fuel targets.

11. **Effects of Heavy Metals and Produced Water on Microalgae Productivity.** E. Torres, B. McNeil, D. Hess, and J.C. Quinn, Utah State University, USA.

Microalgae represents a promising biofuel feedstock. Currently, cultivation systems are assumed to seamlessly integrate with various waste streams, including industrial flue gases, wastewater systems, and produced water from the oil and gas industry. The composition of produced water and industrial flue gases could contain growth inhibiting contaminants, such as heavy metals. This study focuses on the effects of individual heavy metals (As, Cd, Cr, Co, Cu, Pb, Ni, Hg, Se, and Zn) and produced water on microalgae productivity. Produced water was obtained from the oil and natural gas industry in the Uintah Basin, Utah. Results show that productivity is greatly reduced when grown in produced water. In contrast, microalgae grown in the presence of most heavy metals experience minimal effects. However, Ni proved to be detrimental to growth at all tested concentrations, and Cu had negative effects when the concentration exceeded 1.3mg/L. In addition, ICP-MS analysis showed that Cd, Co, Cu, Pb, and Zn were mostly sorbed by the biomass, while As, Cr, Ni, and Se exhibited lower sorption. Results highlight the need for improved understanding of the integration of microalgal cultivation with industrial waste streams.

12. **Soy-oil-based Waterborne Polyurethane Improved Wet Strength of Soy Protein Adhesives on Wood.** H. Liu, C. Li, and X.S. Sun, Bio-Materials & Technology Lab., Dept. of Gran Science & Industry, Kansas State University, USA.

Soy-oil-based waterborne polyurethane (WPU) is used to improve wet adhesion of soy protein isolate (SPI) by dispersing WPU into SPI slurry. WPU's effects on the physiochemical properties of WPU-SPI adhesives are characterized through Fourier Transform Infrared Spectrum, Transmission Electron Microscopy, thermal analysis, contact angle, and mechanical strength. Wet adhesion of the WPU-SPI adhesives increases by 65% compared to SPI control. Moreover, the microstructure of WPU has effects on the interactions between WPU and SPI. In this study, smaller and more uniform distributed WPU0002 is easier to interact and form stronger crosslinking network with protein than WPU0500. The stronger interaction between WPU0002 and protein results in increased viscosity and adhesion strength. The WPU-SPI blended adhesives show significantly improved wet adhesion, demonstrating their potential as wood adhesives.

13. **Oxidatively-cured Coatings from Renewable-based Poly(vinyl ether)s.** D.J. Kalita, M. Sibi, and B.J. Chisholm, North Dakota State University, USA.

Recently, extensive research has been devoted to the development of coatings derived from renewable materials. For this study, vinyl ether monomers were prepared from soybean oil, eugenol, guaiacol, and syringol and a variety of hompolymers and copolymers produced using cationic polymerization. Since each polymer produced possessed pendant allylic and/or bis-allylic groups, they were utilized to produce thermoset coatings by oxidative curing. Structure-property relationships were determined and the results compared to coatings based on commercially available long oil alkyds. It was shown that the properties of the biobased poly(vinyl ether) coatings could be readily tailored by using copolymerization of the different biobased vinyl ether monomers. Coating properties varied over a wide range depending on the chemical composition of the biobased vinyl ether monomers, as well as their relative concentration. In general, the biobased poly(vinyl ether)s showed far superior solvent resistance, film hardness, and hydrolytic stability than the commercial alkyds, while maintaining good flexibility and impact resistance.

14. **Novel Amphiphilic Poly(Vinyl Ether)s Based on Soybean Oil.** K.M. Kingsley[1], S. Samanta[1], S. Stafslien[2], L. Vanderwal[2], and B.J. Chisholm[1], [1]Coatings & Polymeric Materials, North Dakota State University, USA, [2]Research & Creative Activity, North Dakota State University, USA.

Environmentally friendly amphiphilic copolymers were synthesized using cationic polymerization of a vinyl ether monomer derived from soybean oil with different poly(ethylene glycol) (PEG) vinyl ethers. The soybean oil vinyl ether monomer, 2-(vinyloxy)ethyl soyate (2-VOES), was prepared by base-catalyzed transesterification of 2-(vinyloxy)ethanol and soybean oil. An important feature of this type of polymerization is the ability to polymerize exclusively through the vinyl ether double bond, while still maintaining unsaturation from the fatty acid ester pendent groups derived from the soybean oil starting material. Copolymers were synthesized by cationic polymerization using varying weight ratios of 2-VOES and PEG vinyl ethers that varied with respect to the number of ethylene glycol units. Coatings were produced on steel substrates and cured at ambient conditions through autoxidation. The properties that were analyzed included drying time, solvent resistance, hardness, impact resistance, adhesion, contact angle, and flexibility. Free films were also produced and used to measure mechanical and viscoelastic properties of the copolymers. Additional analysis of free films included AFM, protein absorption, water absorption, and red blood cell hemolysis.