BIOTECHNOLOGY

BIO 1: Biocatalysis I — Oleochemicals and Novel Bioprocesses

Chairs: Ching Hou, Retired USDA, USA; and Jun Ogawa, Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan

Developing Foods to Address Health Concerns throughout the Lifespan Kaori Nakajima*, *The Nisshin OilliO Group, Ltd., Japan*

"The Nisshin OilliO Group is committed to contributing to healthy and happy lifestyles. Through the unlimited potential of plant resources and our cutting-edge technology, we promise to lead in the creation of products and services that meet our customers' needs and make a contribution to society." This is the Core Commitment of the company. And it is what inspires us to pursue solutions for the health and happiness of those in need. Founded in 1907, the Nisshin OilliO Group, Ltd., is a 111-year-old company with corporate headquarters in Tokyo, Japan and manufacturing facilities in Japan, Spain and Malaysia and is the largest edible oil company in Japan. The company is divided into several business domains: The Oil and Meal Business, which offers edible oils for household, commercial and food processing applications as well as oilseed meals and soy foods; the Processed Oils and Fats Business which offers margarines, shortenings, processed oils and fats and specialty fats; and the Fine Chemicals Business which sells raw materials for cosmetics, medium-chain triglycerides (MCTs), food additives and chemical products. There is one other business domain, small but mighty: The Health Science Business. It weaves a vertical thread through the large business domains and unites the "health" and "science" aspects of the whole food and oil production to address health concerns throughout the lifespan. The major role of the Health Science Business is to oversee development of food products containing MCTs for the prevention of lifestyle-related diseases, for the elderly and people in nursing care homes and for people with certain medical conditions. Nisshin OilliO has been producing and intensively studying MCT oil since the early 1970s, well before awareness of its very existence and its many potential benefits were widely known. Committed to research, we have explored the use of MCTs for promoting weight loss; providing instant energy as ketones to fuel the brain; preventing lactate build-up during exercise; helping management of certain disease states, such as epilepsy (MCT oil modified ketogenic diet), Alzheimer's disease, autism; reducing risk factors for heart disease; the lowering of blood glucose, which could help with control of diabetes when incorporated into the diet; and applications related to MCTs' anti-microbial effects. Nisshin OilliO endeavors to create a variety of new foods containing MCTs that are designed to appeal to a broad range of tastes and that are adapted to special needs of specific age groups. The MCT puddings, for example, are easy to swallow and come in a satisfying portion packaged in a shallow cup to provide a small tasty snack or dessert. While most MCT oil products on the market contain a roughly 60%/40% combination of C8 to C10, we studied various ratios and found that a higher proportion of C10 to C8 was better tolerated and, therefore, would be more suitable in a product not only for the elderly, but also for anyone who might be sensitive to the standard MCT oil ratio.

Harnessing the Oil Palm Genome for Enhancing Fatty Acid and Carotenoid Composition Rajinder Singh*, Eng-Ti Leslie Low, Meilina Ong-Abdullah, Mohd Din Amiruddin, Mohamad Arif Abd Manaf , and Ghulam Kadir Ahmad Parveez,*Malaysian Palm Oil Board, Malaysia*

The Malaysian Palm Oil Board (MPOB) has sequenced the genomes of both species of oil palm namely *Elaeis guineensis* and *Elaeis oleifera*. More recently, improved gene models were published for oil palm. The gene model improvement was focussed on the fatty acid genes, as part of the endeavour to understand why *E. oleifera* has more unsaturated oil than the commercially planted *E. guineensis*. Increasing the levels of unsaturation, mainly the monounsaturated oleic acid, is desirable. Palm oil with high levels of oleic acid can be more industrially useful for producing oleochemical derivatives, a superior alternative to petrochemical feedstocks. Both molecular breeding and gene technology are as such, being applied to develop planting materials with a higher content of the unsaturated fatty acids, especially oleic acid. Significant progress has been made in this direction using the molecular based tools. The commercial importance is clear, as biorefineries producing oleochemicals have long been established in Malaysia. MPOB has also developed a refining technology that does not result in thermal decomposition of carotenoids, hence making it possible to prepare carotene rich palm oil known as red palm oil. The availability of technology to produce carotenoid rich palm oil has also provided the impetus to breed for oil palm, especially *E. guineensis* or interspecific hybrids (*E. guineensis* x *E. oleifera*) with increased carotene composition. The available reference genome builds with high quality gene models are providing the necessary tools to expedite the biotechnological attempts to increase the value of economic products from oil palm.

Production of Palmitoleic Acid-rich Triacylglycerols by Saccharomyces cerevisiae to Control of Skin Microbiome Toshihiro Nagao*[1], Kazue Narihiro[2], Shimemitsu Tanaka[1], Kazuyoshi Kimura[3], Kazuhiro Yamashita[2], and Yasushi Kamisaka[4],[1]*Osaka Research Institute of Industrial Science and Technology, Japan;* [2]*YAEGAKI Bio-industry, Inc., Japan;* [3]*National Institute of Advanced Industrial Science and Technology, Japan;* [4]*Bioproduction Research Institute, AIST, Japan*

Atopic dermatitis (AD) is a chronic inflammatory skin disease caused by allergens, genetic factors, stress, and skin microorganisms. Since much attention has been focused on human commensal microorganisms, we focus on two skin microorganisms, Staphylococcus aureus and epidermidis. A pathogenic bacterium, S. aureus, is rarely observed in healthy control's skin, but a good bacterium, S. epidermidis, is observed more than S. aureus, and produces several S. aureus growth inhibition factors. Sapienic acid (SA, 6-cis-C16:1) observed in human skin lipid shows selective antibacterial activity: strong activity against S. aureus and weak activity against S. epidermidis at acidic condition. However, in AD, SA content decreases to about one tenth and S. aureus increases followed by aggravation of inflammation. So, supplementation of SA to AD patient's skin should be effective. However, SA rarely observe in natural oils. We thus found that palmitoleic acid (POA, 9-cis-C16:1) in several natural oils showed the same selective antibacterial activity as SA. Kamisaka et al. showed that Saccharomyces cerevisiae produced high POA content (51%) triacylglycerol (yeast TAG) through gene recombination and modification of cultivation conditions. However, oleic acid (OA, 9-cis-C18:1) contaminated in the yeast TAG (OA, 32%) inhibited antibacterial activity against S. aureus. In this study, we found that the activity inhibition level by OA was dependent on S. aureus strains. Furthermore, we studied about lipase-mediated decrease of OA in the yeast TAG, and construction of a new novel Saccharomyces cerevisiae strain producing high POA and low OA content TAG.

Molecular Breeding and Characterization of an Oleaginous fungus *Mortierella alpina* for a Prostaglandin, $PGF_{2\alpha}$, Production Jun Ogawa*[1], Mohd Fazli Farida Asras[2], Hideaki Nagano[2], Yoshimi Shimada[2], Miho Takemura[3], Shigenobu Kishino[4], and Akinori Ando[2],[1]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan;* [2]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan;* [3]*Res. Ins. Biore. Biotech., Ishikawa Pref. Univ., Japan;* [4]*Kyoto University, Japan*

One of the prostaglandins, $PGF_{2\alpha}$, is generated from arachidonic acid via the cyclooxygenase (COX) reaction. $PGF_{2\alpha}$ is a vital bioactive molecule important in pharmaceutical industries and playing an important role especially in the regulation of physiological processes such as blood circulation. However, $PGF_{2\alpha}$ is limited in nature and has been widely synthesized chemically, yet hardly been produced by any biological process especially in microorganisms. The author constructed a binary vector which contains the COX gene from *Gracilaria vermiculophylla* (GvCOX) regulated by a constitutive promoter, His550 with a modest expression level, and introduced it to an arachidonic acid-producing *Mortierella alpina* 1S-4 by the *Agrobacterium tumefaciens*-mediated transformation method. The transformants obtained was capable to produce $PGF_{2\alpha}$ extracellularly by fermentation. The transformants with the stronger SSA2 promoter, which consistently increased the expression of GvCOX until the end of the fermentation period, produced much more $PGF_{2\alpha}$. The optimization of several fermentation conditions, such as concentration of the initial glucose and the addition of free fatty acid, resulted in the further enhancement of $PGF_{2\alpha}$ production.

Co-production of Carotenoids and Polyhydroxyalkanoates by Paracoccus sp. LL1 Beom Soo Kim*[1], Prasun Kumar[2], Won-Gyun Oh[2], and Mehtab Muhammad[2],[1]*Chungbuk National University, Republic of Korea;* [2]*Chungbuk National University, South Korea*

Polyhydroxyalkanoates (PHA) have gained much attention as an alternative to synthetic plastics in the past decades owing to their biological origin, comparable thermoplastic properties, and biodegradable nature. Possible methods to reduce the high production cost of PHA are to use inexpensive substrates such as lignocellulosic biomass and industrial effluents, e.g. crude glycerol, wastewater, etc. Additionally, co-production of various valuable bioproducts along with PHA has been proposed to alleviate the overall production cost. This study demonstrates that PHA and astaxanthin-rich carotenoids can be co-produced by Paracoccus sp. LL1 using various substrates such as glucose, methanol, lactose, galactose, glycerol, fructose, mannitol, and valerate. The type of co-produced PHA was found to be poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB/3HV) copolymer from FTIR and NMR analysis. In batch culture of Paracoccus sp. LL1 using 1% valerate as a carbon source, PHA accumulation of 39% of DCW was observed at 96 h with very high 3HV content of 95 mol% (3.2 g/L of DCW) and carotenoid concentration of 12 mg/L. Cell retention culture of Paracoccus sp. LL1 using glycerol resulted in DCW of 24 g/L containing 39% PHA with concomitant production of carotenoids (7.1 mg/L).

Gas-to-Lipids Bioprocessing by Acetogens and Thraustochytrids Charose MT Perez[1], Ran Hirotani[1], Motomu Ishigaki[1], Kenshi Watanabe[1], Yoshiko Okamura[1], Takahisa Tajima[1], Yukihiko Matsumura[1], Yutaka Nakashimada[1], Yusuke Sumita[2], Shinzo Mayuzumi[3], and Tsunehiro Aki*[1],[1]*Hiroshima University, Japan;* [2]*Chugoku Electric Power, Japan;* [3]*Idemitsu Kosan, Japan*

There has been a collective effort in reducing greenhouse gas emissions globally, and scientist are in the process of developing

technologies to do so. We have previously established composite fermentation systems for producing useful lipids from various biomass using two kinds of microorganisms having different metabolic systems. These researches led us to develop conditions where the oleaginous microorganism, *Aurantiochytrium* sp., shows high assimilability of organic acids, especially acetic acid. We then focused on acetogens, which release acetic acid as the main product of their metabolism, to examine its combination in lipid fermentation. Acetic acid fermentation with *Acetobacterium woodii* was performed using carbon dioxide as a substrate, and the culture broth was successfully utilized by *Aurantiochytrium* sp. for efficient production of glycerides. Furthermore, the metabolic profile of lipid fermentation was subjected to the metabolome analysis to extract improvement factors for optimization of this new gas-to-lipids bioprocess.

Hydrolysis of Raffinose in Complex Soybean Waste by Engineered *P. chlororaphis* as Biocatalyst Daniel K.Y Solaiman*, Richard D. Ashby, and Nicole V. Crocker,*USDA, ARS, ERRC, USA*

Soybean processing and utilization industries make good uses of the high-value products such as the soybean oil and the soybased proteins for food and non-food applications. The low-value byproducts such as the soy molasses (SM) and tofu whey (TW) however still contain sugars useful for fermentation processes to produce high-value microbial natural products. This paper reports the genetic engineering and testing of *Pseudomonas chlororaphis* to grow and utilize the sugars in culture media supplemented with SM or TW as carbon source. *P. chlororaphis* was chosen for the study because it can synthesize both a biopolymer (i.e., polyhydroxyalkanoates) and a biosurfactant (i.e., rhamnolipids). A two-stage fermentation process was described for testing the hydrolysis of galacto-oligosaccharide by the *P. chlororaphis* recombinants. Results showed that the recombinant *P. chlororaphis* strains effectively hydrolyze raffinose (a galacto-oligosaccharide) in SM and TW, and subsequently metabolize the resultant galactose in the medium. The genetically engineered *P. chlororaphis* strains are therefore potentially useful for production of biobased products using the low-cost SM and TW feedstocks.

Synthesis of Trimethylolpropane Triester Using an Immobilized Lipase in a Recirculated Packed Bed Reactor Heejin Kim[1], and In-Hwan Kim*[2],*[1]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea; [2]Korea University, Republic of Korea*

Synthetic oleochemical esters of polyols and fatty acids are biodegradable and possess desirable technical and ecological properties. Trimethylolpropane (TMP) triester has been widely applied as a hydraulic fluid. TMP triester was effectively synthesized by lipase-catalyzed esterification from TMP and high oleic fatty acid from palm oil in a recirculating packed bed reactor (RPBR). The immobilized lipase was prepared with liquid Eversa Transform 2.0 from Thermomyces lanuginosus with Lewatit VP OC 1600 as a carrier. The effects of temperature, residence time of substrate in RPBR, and vacuum level on the synthesis of TMP triester were investigated. The optimum temperature, residence time of substrate in RPBR, and vacuum level were 70 °C, 1 min, and 20.0 kPa, respectively. Under the optimum conditions, the maximum conversion reached up to 85% after 6 h.

BIO 1.1/IOP 1: Biopolymers

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

Plant Oil Derived Emulsion Copolymers with Predictable Properties Meghan E. Lamm*, Ping Li, and Chuanbing Tang, *University of South Carolina, USA*

Emulsion polymerization is an important industrial synthetic method. Unfortunately, it relies mostly on petroleum-based chemicals. Recent research has focused on incorporating biomass derived chemicals and intermediates, such as plant oil-based monomers. Due to their good hydrophobicity, plant oil monomers can struggle to be incorporated into emulsion polymers to achieve optimal properties. Herein we present an industrially relevant semi-batch method of emulsion polymerization used to prepare copolymers of soybean methacrylate (SBMA), using high oleic soybean oil (HOSO), with various industry co-monomers such as methyl methacrylate, styrene, and butyl acrylate. All polymerizations displayed good control with minimal coagulation and high conversion. Variation in feed ratios (from 0 to 50 wt% of SBMA) offers tunability of thermal and mechanical properties in resulting latex materials. The alkene present on the soybean chains allowed for oxidative crosslinking of latexes films, which resulted in high levels of crosslinking, consistent with SBMA content, and significant enhancement in mechanical properties. Overall, the promising results indicate good potential for replacement of petrochemical methacrylates in commodity thermoplastics and acrylic coatings.

Corn Oil for Highly Flame Retardant Rigid Polyurethane Foams for Industrial Applications Camila Zequine, Sanket Bhoyate, Brooks Neria, Pawan Kahol, and Ram Gupta*, *Pittsburg State University, USA*

Vegetable oils are being used as starting materials for the synthesis of various biopolymers. Polyurethanes are an important class of polymers because of their wide industrial applications in consumer and industrial sectors. The main disadvantage of the polyurethane foams is their high flammability which is due to the predominant presence of carbon and hydrogen in their structure. Highly porous and combustible nature of polyurethane foams further facilitates the flame spread rate. High flammability of polyurethane foams restricts some of its valuable applications. In this work, we have used corn oil as a starting material for the synthesis of polyurethane foams. Corn-oil was converted to polyol for polyurethane foams using “thiol-ene” click chemistry. Flame-retardant polyurethane foams were prepared by addition of different concentrations of dimethyl methyl phosphonate in the final foam composition. The effect of additive flame retardant on the physicomechanical and flammability of the polyurethane foams was studied. Additive flame retardant significantly reduced the flammability of the polyurethane foams without affective their physicomechanical properties. The foam containing flame retardant showed over 30 times reduction in the burning time and over 6 times reduction in weight loss during horizontal burning test compared to foam without the flame retardant. In addition to reducing the burning time and weight loss after the addition of the flame retardant, these foams showed a significant reduction in heat release rate and thermal heat release. Our research opened a new pathway to utilize vegetable oils for industrial applications, particularly in the polyurethane industry.

Rapid Conversion of Oils into Various Monomers and Biopolymers Aman Ullah*, Muhammad Arshad, Reza Ahmadi, and Liejiang Jin, *University of Alberta, Canada*

The use of renewable resources in

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

Cationic Polymerization of Epoxidized Oils to Cast Resins and Foams Zoran Petrovic*, and Dragana Radojcic, *Pittsburg State University, USA*

Epoxidized oils are an excellent platform for a range of new products. Here we describe the use of bulk polymerization of epoxidized soybean oil, linseed oil and triolein to obtain resins useful as encapsulants, potting compounds, and foams based upon using different cationic initiators. While super acids and Lewis acids were preferable in making foams because they enhance reaction rates, novel catalysts were used to slow down and control the polymerization rate and obtain solid polymers with unusual properties.

Synthesis, Properties and Structure Function Correlation of Bioplasticizers in PVC. Dharma R. Kodali*, and Lucas J. Stolp, *University of Minnesota, USA*

This presentation provides an overview of the synthesis, properties and functional evaluation of bioplasticizers in polyvinylchloride (PVC) that are derived from vegetable oils. Plasticizers, the largest class of plastic additives, are non-volatile organic liquids that impart flexibility and improve the functionality of plastics. The currently used phthalate plasticizers have EHS concerns. Bioplasticizers with various functional groups such as epoxy, acetoxy, methoxy, thiirane, aziridine on the acyl chain backbone combined with ester group variations of alkyl, mono, di and tri alkoxy or solketal of fatty acid esters have been synthesized and characterized. The bioplasticizers were derived from regular and high oleic soybean, canola and castor oils, and formulated with PVC and evaluated for their functional properties. Epoxysoybean oil fatty acid esters served as the key intermediate to incorporate most of the functional groups on the fatty acid backbone. The ring opening of the epoxy group with acetic acid resulted in cyclic tetrahydrofuran derivatives. The high viscosity and darker color of aziridine and thiirane derivatives limited their usefulness, whereas the other compounds physical and analytical properties such as acid value, color and viscosity, were acceptable. The plasticizers having epoxy and acetoxy groups demonstrated excellent compatibility in PVC, with high efficiency (Shore Hardness), thermal stability

and gelling properties. The structural variations of molecular weight, polarity, and branching of the fatty acid esters and their effect on functionality is examined and rationalized. The functional properties of number of bioplasticizers were comparable to commercial plasticizer, diisononylphthalate (DINP).

Synthesis and Characterization of Lipid-based Biopolymers and Bionanocomposites from Poultry Industry By-product Muhammad Safder*, *University of Alberta, Canada*

Spent hen is a by-product of the poultry industry and has potential as a source of lipids. Lipid-based monomers and corresponding polymershave successfully been synthesized by using free radical polymerizations. The effect of temperature and time on molecular weight of polymer was studied and conditions for obtaining high molecular mass biopolymers were optimized. Furthermore, using optimized conditions, the biopolymers were reinforced with different nanoparticles such as nanoclay, POSS, and monocrystalline cellulose (NCC) using in-situ polymerizations. The monomer, homopolymer and nano-reinforced polymers were characterized by different techniques such as nuclear magnetic resonance (NMR), attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), tensile testing and dynamic mechanical analysis (DMA). The thermal properties of the synthesized materials were evaluated by the thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The nanofiller addition into the polymer matrix substantially improved the thermal and mechanical properties of the composites.

Corn Stover and Levulinic Acid: Two Valuable, Renewable Substrates for Biosynthesis of Unique Polyhydroxyalkanoate Biopolymers Richard D. Ashby*, Daniel K.Y Solaiman, Gary Strahan, and Alberto Nunez, *USDA, ARS, ERRC, USA*

Lignocellulosic materials are abundant and cheap and as such, are being evaluated as feedstocks for bio-based product synthesis. Our laboratory used the hydrolysate from corn stover (CSH) as a base fermentation feedstock for the synthesis of polyhydroxyalkanoate (PHA) biopolymers using *Burkholderia sacchari* DSM 17165 and *Azohydromonas lata* DSM 1122. *B. sacchari* utilized all of the available sugars in the hydrolysate while *A. lata* only used the glucose fraction of the hydrolysate to produce poly(3-hydroxybutyrate) (PHB). Introduction of levulinic acid (another inexpensive bio-based material produced from both C-5 and C-6 sugars) into the media in a co-feeding strategy resulted in block copolymers composed of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV; P3HB-3HV) from *B. sacchari* and terpolyesters composed of 3HB, 3HV, and 4-hydroxyvalerate (4HV) from *A. lata* with varying monomer ratios. This presentation will focus on the specific details of polymer production and characterization protocols involved in the synthesis of these unique PHA biopolymers.

Microalgae for the Production of Novel Biopolymer Feedstocks Scott Franklin*[1], Zoran Petrovic[2], Jian Hong[3], Leon Parker[1], Lauren Slutzky[1], Mona Correa[4], Nina P. Reyes[1], Constantine Athanasiadis[4], Jon Wittenberg[1], Estelle Schaefer[5], and Kevin Ward[1],*[1]Checkerspot, USA; [2]Pittsburg State University, USA; [3]Kansas Polymer Research Center, Pittsburg State University, USA; [4]Checkerspot; [5]Checkerspot, France*

The oleochemicals industry represents an enormous market valued at greater than $23B U.S. that is responsible for the production of myriad products, materials and polymers via processing of fats and oils derived from animal and vegetable sources. Surprisingly, this

industry relies on just 14 fatty acids to create these chemical building blocks, because these fatty acids are what are readily available at commodity scale and price. On the other hand, plant oilseeds found in nature, most of which will never be cultivated at industrial scale, elaborate an incredible diversity of fatty acid moieties (> 500 species) unavailable in today's oleochemicals industry. The chain lengths, degrees of saturation, functionalities and purity of these fatty acids and their resulting triglycerides offer unique opportunities for the production of polymer feedstocks, particularly with regard to polyols for PU applications. Microalgae, which elaborate fatty acids and triacylglycerols utilizing cellular machinery homologous to that found in higher plants, have proven to be highly efficient microbial factories for the production of biomass, proteins and oils, and many species appear to be quite amenable to genetic manipulation as well as to standard techniques of microbial strain improvement. Utilizing these tools and combining whole genome and transcriptomic sequencing of unique oilseeds and host microalgae, novel triglycerides can be produced in these single-cell factories at exceptionally high purity and titer, exceeding what is present even in the natural oilseed host. Data gleaned from materials (foams, elastomers and cast urethanes) produced utilizing the above approach will be shown.

BIO 1.2a/PRO 1a: Advances in Enzyme Processing Technologies

Chairs: Long Zou, Bunge Oils, USA; and Leslie Kleiner, Roquette Americas Inc., USA

Unique Phospholipase Degumming Enzyme Michael E. Spampinato*, *DSM Inc., USA*

As an oilseed processor you are faced with the continued challenge of improving output and reducing costs whilst remaining environmentally friendly. Our Purifine 3G enzymes can make a big difference to your oilseed extraction process, crushing the seeds more effectively, hiking your yields, increasing the value of your meal, all leading to a boost in your profit margins. These enzymes work as catalysts to break phospholipids into water-soluble and oil-soluble fragments breaking the emulsion formation and making degumming easier.

Support Areas to Example:

- Higher oil yields thus more profit:
- How Purifine 3G works
- Higher meal value, reduced protein dilution
- Easy to integrate, consistent results
- First-time right performance

New Enzymatic Process Improves the Yield in Alkaline Refining of Vegetable Oils Hans Christian Holm, and Per Munk Nielsen*, *Novozymes A/S, Denmark*

Use of enzymes in the degumming process is accepted in the vegetable oil processing. The effect of the enzymes is first and foremost to obtain a highest possible yield, but also to help assuring a good quality of the refined oil. The known processes use different types of enzymes. In the water degumming process extra yield is obtained by a treatment with phospholipase C/A, and in acid refining use of phospholipase type A is the typical solution. In the alkaline refining process, the phospholipids are removed from the oil by a water degumming and the caustic treatment. This comes with a yield loss. We have identified a new way of operating the alkaline refining process by integrating a phospholipid hydrolysis into the process line. It has been possible to design an alkaline refining process where the enzyme treatment with phospholipase C fits in, and results in a significant yield increase. The process includes following steps: an acid chelating, pH adjustment, enzyme reaction, alkaline treatment, separation, and washing. The process will be presented with the documentation for the yield improvement compared to a typical chemical refining process.

Enzymatic Interesterification. Chris Dayton, Bunge, USA

Kinetic Modelling of Enzymatic Saccharification of Soy Molasses Ashwin Sancheti*, and Lu-Kwang Ju, *University of Akron, USA*

Soy molasses is a low-value stream generated in the soybean industry. It is primarily composed of stachyose and sucrose. The enzymatic hydrolysis of soy molasses produces a rich feedstock composed mainly of monomeric sugars glucose, galactose and fructose, which can be used for multiple biotechnological applications such as arabitol production by *Debaryomyces hansenii* and fatty acid synthesis by engineered *Escherichia coli* (collaboration with Drs. Ka-Yiu San and George Bennett of Rice University). The enzyme used was produced by *Aspergillus niger* using soybean hulls as substrate. The objective of this study was to model and optimize the enzymatic hydrolysis of stachyose and sucrose in soy molasses, using a semi-mechanistic, multi-reaction network to describe the kinetic profiles of reactants, intermediates and products measured by the HPLC-RI analysis. The model predicted monomeric sugar release with reasonable accuracy and can help in

formulating the optimized cocktail of enzymes to produce by adjusting *A. niger* fermentation conditions. Results showed that the enzyme composition impacts the reaction pathways, leading to different profiles of intermediates and side products. The interactions among different assayed enzyme activities will be discussed in the presentation.

BIO 2: Biocatalysis II — Functional Foods and Natural and Derived Oleo-materials

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

Restructuring Lipids for Functionality and Health Casimir C. Akoh*, *University of Georgia, USA*

Natural occurring lipids were restructured, with the aid of enzymes, for improved functionality in food applications and with potential nutritional benefits. These include the production of flavor esters, trans-free fats, reduced calorie or low saturated fats, cocoa butter alternatives, phenolipids as antioxidants, and infant formula fat analogs. Some of the designed lipids served as oil substrates to form oleogels/organogels. Organogels can serve as alternatives for high saturated fats and trans fats. Encapsulated was used to protect the sensitive unsaturated fatty acids from oxidation. Desirable attributes of fats and oils for functionality in foods include oxidative stability, fatty acid profile, crystal and polymorphic form, melting property, solid fat content, n-3/n-6 ratio, emulsification property, and flavor. The overall aim of our research is to improve human health and to show the connection between food science and nutrition. Restructured lipids may play a role in immune function modulation, improve blood lipid profile, enhance absorption of sn-2 fatty acids, and reduce cholesterol, cancer, and heart disease. In the case of infants, they may help improve absorption of calcium and lipids, lead to proper growth, brain function, mental, neuronal and cognitive developments. The design or restructuring to produce any specific lipid structure is an art that benefits from a good understanding of chemistry, biochemistry, metabolism, physiological function, nutritional, and functional properties of lipids.

Improvement in Enzymatic Enrichment of DHA in Algal Lipids by Thermostable Lipase Preparation. Yomi Watanabe*[1], Tsunehiro Aki[2], and Araki Masuyama[3],[1]*Osaka Research Institute of Industrial Science and Technology, Japan;* [2]*Hiroshima University, Japan;* [3]*Osaka Institute of Technology, Japan*

Marine algae is one of the alternative natural sources of polyunsaturated fatty acids. The oil produced by *Auranthiochytrium* sp., is uniquely rich in PUFA, especially in 22:5 (DPA) and 22:6 (DHA). We have previously reported that enzymatic treatment of fish oil with ethanol/water mixture is efficient to increase DHA content in acyl glycerol fraction. DHA content in sardine oil was increased from 13% to 45-50% DHA with 70-80% recovery by the *Thermocyces* sp. liquid lipase (Eversa, Novozymes) treatment of the oil in 20% ethanol solution at 35°C. DHA content *Auranthiochytrium* oil from 37% to 63% required at 45°C reaction, but with 4 times more lipase amount due to the higher melting point of oil. The recent lipase preparation, version 2.0, showed higher temperature stability than the previous one and achieved the similar DHA concentration rate with half amount of lipase.

Interesterification of Palm Based Oils for Specialty Fat Hardstock: Comparison of Enzymatic Catalysis and Chemical Catalysis Jing Ye[1], Zhen Zhang[2], Ying Li[3], and Yong Wang*[1],[1]*Jinan University, China;* [2]*South China University of Technology, China;* [3]*Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, China*

Interesterification as an effective oil modification method has been widely used in the production of specialty fats in food. The blend of palm olein, palm kernel oil and palm

stearin with mass ratio of 5:3:2 was interesterified using sodium methoxide and Lipozyme TL IM catalysts. The comparison of the solid fat content profiles, thermodynamic properties, crystal polymorphism and microstructures, as well as crystallization kinetics of the blends between chemical (CIE, catalyst loading 0.3%, reaction temperature 105 oC and time 30 min) and enzymatic interesterification (EIE, 800 g enzyme in a pilot-scale packed bed reactor, reaction temperature 60 oC and time 15 min) was investigated. The solid fat content of EIE samples ranging from 10 to 40 oC, as well as viscosity and hardness, were below the CIE samples. which was consistent with rheology results. Compared to CIE samples, EIE can significantly expedite crystallization rate at 10 oC. The higher value of Avrami constant K also suggested that the crystallization rate of EIE samples was faster than CIE samples, the exponent n indicated that the mechanisms of nucleation and growth of EIE was more rod-like growth with instantaneous nuclei. β and β′ forms were both observed in the X-ray diffraction spectra of all interesterified samples. However, the microstructure indicated that a finer crystal structure appeared after EIE modification. The physical characteristics of EIE products exhibited improved crystallization behaviors with better structure and lower hardness, which proved the potential of EIE in plastic fats applications.

Efficient Production of MLCT Oils by Lipase Reactions Yutaro Kataoka*, Hidetaka Uehara, and Yoshihiro Ueda, *The Nisshin OilliO Group, Ltd., Japan*

Lipase catalyzed inter-esterification is an excellent method to make functional structure lipids. It can be conducted at normal temperature and pressure, so deterioration of reactants can be reduced and side reaction can be controlled. Moreover, characteristic structure lipids can be created by lipase's position specificity of esterification. Examples of practical application include cacao butter equivalent for the purpose of improving physical properties and medium and long chain triacylglycerol (MLCT) for nutritional improvement. However, the inter-esterification reaction using lipase has been practically used only in part. One of the major reasons is the cost. Since a decrease in lipase stability leads to an increase in cost, maintaining stability during prolonged reaction is a major subject. However, enzymatic reactions are delicate, so there are hurdles to fix the proper reaction system. In these issues, we examined from two points, with a view to manufacturing MLCT oils. At first, we searched lipases suitable for production. We compared reactivity of lipases from commercialized to original ones, and we identified stable lipases. Secondly, we tried to suppress the decreasing lipase stability by optimization of reaction system. Specifically, we adjusted reaction conditions and reaction mode (batch or continuous) thereby establishing a stable reaction system. We also clarified that lipase stability is maintained by pretreating the reaction substrate. From the above studies, we were able to enhance the lipase activity and achieve the design of stable production system. As a result, we have been able to manufacture a high quality MLCT oils with low cost.

Synthesis of Triacylglycerol Containing Hydroxy Fatty Acids as a Constituent Fatty Acid Shigenobu Kishino*[1], Daichi Toyama[1], and Jun Ogawa[2],[1]*Kyoto University, Japan;* [2]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan*

[Introduction] 10-hydroxy-*cis*-12-octadecenoicacid (HYA) produced via saturation metabolism of linoleic acid to oleic acid in lactic acid bacteria has an intestinal barrier function and an effect of suppressing triacylglycerol (TG) accumulation in the liver. There is a possibility that HYA is useful for preventing obesity. Since

free form fatty acids are peculiar pungent, it is desirable to produce HYA in the TG form. In this study, production of TG containing HYA as a constituent fatty acid was studied using lipase applicable for food manufacturing process. [Results and discussion] Acidolysis reaction catalyzed by lipase was applied to HYA-containing TG production. Free fatty acids that contained about 30% hydroxy fatty acids were used for the acidolysis reaction. Lipases applicable for food manufacturing process (4 kinds of powder, 1 type of immobilizing) were evaluated. Furthermore, we attempted to immobilize powder lipases to several types of resin. We also evaluated the acidolysis activity in repeated reaction, the effects of edible oil, the ratio of edible oil and lipase, and reaction temperature on the acidolysis activity. As a result, using the immobilized microbial lipase derived from *Rhizomucor miehei*, the HYA content in TG reached to about 35% under the optimized reaction conditions.

Production of xanthophylls by New Zealand Microalgae and the Sea Urchin Evechinus chloroticus (Kina) Donato Romanazzi*[1], Johnathon Puddick[1], Masashi Hosokawa[2], Matthew R. Miller[3], Michael Packer[3], Serean Adams[1], Ruihana Paenga[4], and Sarah Bond[5],[1]*Cawthron Institute, New Zealand;* [2]*Hokkaido University, Japan;* [3]*Cawthron, New Zealand;* [4]*Hikiarangi Bioactives Limited , New Zealand;* [5]*Massey University, New Zealand*

Microalgae produce many valuable molecules that can be used as ingredients in functional foods, as nutraceuticals, in cosmetics/cosmeceuticals and as biochemical reagents. Further Kina, a NZ native sea urchin (Evechinus chloroticus) is an underutilised resource and a rich source of bioactive materials. **Objectives** Through an international research collaboration between the Cawthron Institute (NZ) and Hokkaido University (Japan) we tested the ability of the golden-yellow algae Tisochrysis Lutea (T-Iso) to accumulate fucoxanthin and its potential health applications. Additionally, under the National Science Challenge of Sustainable Seas, a research collaboration has formed between Hikiarangi Bioactives Limited, Massey University, Cawthron and local indigenous groups to evaluate the bioactives in Kina across season and location. **Methods** Cawthron has developed methods to improve the yield of target molecules from microalgae and optimised the conditions for growth and xanthophyll accumulation in continuous culture of T-Iso. Chromatographic techniques were used for isolation and purification of fucoxanthin from microalgae biomass. Extracts rich in echinenone were produced and assessed for levels or carotenoids including echinochrome. **Results** A range of xanthophyll rich products, including algal and urchin extracts, have been evaluated in a series in vitro models of inflammation, joint health and an in vivo model of allergy.

Production of ω3-docosapentaenoic Acid (DPA) by *Aurantiochytrium* sp. T7 Strain Akinori Ando*[1], Ayami Hatano[2], Tomoyo Okuda[1], Hiroshi Kikukawa[3], Keisuke Matsuyama[4], and Jun Ogawa[5],[1]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan;* [2]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan;* [3]*Gifu university, Japan;* [4]*NAGASE & CO., LTD., Japan;* [5]*Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan*

Omega-3 polyunsaturated fatty acids (ω3-PUFAs) are found in natural sources. Each ω3-PUFA and its derivative have been reported to have some particular physiological function. EPA, DHA, and ALA, which can be prepared from natural sources, have been well studied because of their sufficient natural supply. On the other hand, there are few reports on the other ω3-PUFAs, such as stearidonic acid (SDA, 18:4ω3), docosapentaenoic acid (DPA, 22:5ω3),

and eicosatetraenoic acid (ETA, 20:4ω3), because their supply has been limited to date. These minor ω3-PUFAs have been expected to possess beneficial function as precursors of bioactive substances as well as EPA and so on, therefore sufficient supply of such rare PUFAs has been required for elucidation of their physiological function. ω3-DPA has been reported to be present in several natural oils obtained from *Phoca groenlandica*, *Cyanea capillata*, and *Mola mola*. But the content of ω3-DPA was no more than 5% in total lipid. Thus, recent investigations have focused on ω3-PUFAs production by alternative source such as oleaginous bacteria, fungi, plants, and microalgae. In this study, we screened Labyrinthulomycetes for ω3-DPA production from brackish water areas in Japan and obtained *Aurantiochytrium* sp. T7 strain which accumulates a significant amount of ω3-DPA. Subsequently, we evaluated the effects of various culture conditions on ω3-DPA productivity of *Aurantiochytrium* sp. T7 strain. As a result, we revealed that *Aurantiochytrium* sp. T7 strain showed 165 mg / L ω3-DPA productivity under optimal conditions (2% Glucose, 1% Yeast extract, seawater concentration 50%, initial pH 5.5, culture period 4-6 days).

PUFA Enriched PG Synthesized by PLD-mediated Transphosphatidylation Exerts Anti-inflammatory Effect upon LPS-stimulated RAW264.7 Cells Liping Chen*[1], Masashi Hosokawa[2], and Kazuo Miyashita[2],[1]*Hokkaido University Faculty of Fisheries Sciences, Japan;* [2]*Hokkaido University, Japan*

Recent year, marine phospholipids have received increasing attention due to bioavailability, phosphatidylglycerol (PG) is seldomly studied due to the low content in natural products, in the present study, PUFA enriched PG was synthesized by transphosphatidylation mediated by phospholipase D. In buffered aqueous reaction system, PG yields from SRPC or EYPC declined dramatically which may be partly correlated to large percentage of LCFA and SFA, and with the addition of α-tocopherol exerted promotion of PG synthesis. Furthermore, PG yields from salmon roe lipid mixture SRTL or crude PL in two reaction systems increased notably compared to purified PC, indicating the endogenous compounds in the lipid mixture may facilitate PG generation, suggesting that the formation of PG from salmon roe lipid mixture mediated by PLD in buffered aqueous reaction system may have favorable application prospect for the preparation of PUFA structured PG. The anti-inflammatory activity of conversed SRPG was investigated by LPS-induced macrophages RAW264.7, the effect could be evidenced by attenuation of the mRNA expression of proinflammatory cytokines, chemokines, enzymes, including IL-6, IL-1β, MCP-1, iNOS, COX-2, and upregulation of antioxidative enzymes including NQO-1, HO-1, inhibition of secreted NO, and suppression of IL-6, IL-1β determined by ELISA. FA accumulation showed that the n-3 PUFA from SRPG could be more efficiently incorporated into cellular lipids, indicating that SRPG with high proportion of PUFA exerted potent antiinflammation activity partly through the alteration of cellular FA composition. Our result suggests that SRPG could improve the bioavailability of n-3 PUFA.

A New Enzymatic Preparation Method for L-α-Glycerylphosphorylcholine for Use as a Food-Grade Cognitive Enhancer Byung Hee Kim*, *Sookmyung Women's University, Korea*

The aim of this study was to prepare L-α-glycerylphosphorylcholine (L-α-GPC) with cognitive-enhancing effects via a lipase-catalyzed hydrolysis of soy phosphatidylcholine or a fractionated soy lecithin, followed by food-grade solvent extraction of L-α-GPC from the reaction products. The reaction was performed

in a biphasic medium in a stirred-batch reactor. Phosphatidylcholine was completely hydrolyzed to L-α-GPC under optimal conditions. Water-soluble fractions of the reaction products containing 98.6 wt% L-α-GPC (from soy phosphatidylcholine) or 52.4 wt % glycerophosphodiesters, including L-α-GPC (from fractionated soy lecithin), were obtained after phase separation of the medium. The resulting products would be suitable for use as food-grade cognitive enhancers due to the use of enzymatic reaction and food-grade solvent extraction.

Roles of Conjugated Linoleic Acids in Oxidation of Vegetable Oils as Functional Lipids Suk Hoo Yoon*, *Woosuk University, Korea*

Conjugated linoleic acids (CLA) have been recognized as a functional lipid due to their ability to prevent or cure cancer, atherosclerosis, and Type II diabetes, to exert a muscle and bone strengthening activity, and to cause fat reduction in humans and animals. The oxidative stability of CLA was almost the same as the stability of linoleic acid (LA) in a bulk phase at high temperature, and soybean oil containing CLA was much more stable than that lacking CLA. The oxidative stability of soybean oil, cottonseed oil, and corn oil was less with CLA than without CLA during autoxidation. The autoxidative stability of oils increased as the CLA content increased. During photooxidation of oils, the oxidative stability of oils was higher with CLA than without CLA, and the stability of oils increased as the CLA content increased. The mechanisms of autoxidation and photooxidation of oils were considered due to the contents and anti- and pro-oxidant activities of individual conjugated linoleic acids in bulk oil, and minor compounds present in oils.

Improved Carbohydrase Production by *Aspergillus niger* Fermentation for Soybean Meal Carbohydrate Hydrolysis for use as Fermentation Feedstock S.M. Mahfuzul Islam*, and Lu-Kwang Ju, *University of Akron, USA*

Enzyme production with proper composition and enriched concentration is the most important and yet challenging criteria in the biorefinery. Soybean meal has great potential of carbohydrate source (30-35%) for producing different chemicals and biofuels. However, owing to the complex structure of carbohydrate (pectin, cellulose, hemicellulose and galacto-oligosaccharides), complex enzyme system with multiple enzyme containing at least pectinase, α-galactosidase, cellulase, xylanase and sucrase is needed. Soybean hull, which is very cheap, was used in this study to use as inducing substrate to produce these carbohydrase enzymes by *Aspergillus niger* fermentation. Different factors have been evaluated in this study to increase the enzyme production. Effect of higher loading of soybean hull was studied from 40–100 g/L at two pH level (pH 6 and 7). To better utilize the different pH dependency of the different enzyme production, three different pH gradient profiles were studied (0.0156, 0.0292 and 0.0357 pH drop per h at 100 g/L soybean hull loading and starting pH 7). All the enzyme production, particularly, pectinase, α-galactosidase and cellulase increased significantly with the increase of pH drop rate. Highest pectinase, α-galactosidase and cellulase activities were found in 0.0357 pH drop per h and activities were pectinase, 19.1 ± 0.04 U/mL; α-galactosidase, 15.7 ± 0.4 U/mL; cellulase, 0.88 ± 0.06 FPU/mL. In this current study, very high enzyme activities have been achieved compared to the previous studies which will make this process very potential to use in soybean meal to produce high monomerized fermentation feedstock for valuable fermentation based product formation.

BIO 3: Plant and Algae Lipid Biotechnology and Genomics

Chairs: Jay Shockey, SRRC-ARS-USDA, USA; and Timothy Durrett, Kansas State University, USA

The interaction of the soybean seed high oleic acid oil trait with other quality traits Kristin Bilyeu*, *USDA/ARS, USA*

Soybean is considered an oilseed, but the value of the crop is comprised of two parts: the vegetable oil and a high protein meal. The utility and nutritional properties of vegetable oil vary due to the fatty acid components of the seed oil. The objective of this research was to determine the interaction of the soybean high oleic acid oil trait in combination with other traits controlling fatty acid profiles. The results demonstrated that targeted stable levels of fatty acids could be achieved across multiple production environments. The impact of the results is identification of the minimum the set of genes for breeders to use to in marker assisted selection that can immediately impact efforts to develop varieties with the oil quality traits that are needed for US producers to be competitive in global markets.

Getting the Most Value Out of Soybeans: A Case for Understanding Resource Partitioning and Allocation Over Seed Development Shrikaar Kambhampati*[1], Jose A. Aznar-Moreno[2], Jennifer J. Arp[3], Sally K. Bailey[3], Kevin L. Chu[1], Timothy P. Durrett[2], and Doug K. Allen[4],*[1]Donald Danforth Plant Science Center, USA; [2]Kansas State University, USA; [3]Donald Danforth Plant Science Center, United States; [4]Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA*

Soybeans, though efficient in protein and oil accumulation, contain a significant (10 % biomass) amount of non-digestible sugars, specifically Raffinose Family Oligosaccharides (RFOs). A comprehensive analysis of metabolite pools in cotyledons over seed development indicate that lipid content decreases towards later stages (R7 to R8), with a corresponding increase in RFOs. It is unclear, however, whether the source of carbon for RFO biosynthesis late in development is lipid turnover via β-oxidation followed by gluconeogenesis, or redirection of glycolytic carbon towards oligosaccharides due to decreased flux in lipid synthesis. While preliminary isotopic labelling studies suggest the latter to be a possible source, current techniques for stable isotope incorporation and measurement are insufficient to quantify fluxes during late stages of seed development. A slow rate of metabolism and large metabolic pool sizes in maturing seed calls for improvement of existing transient labelling strategies, detection techniques and data analyses. In this study, we present novel, rational approaches for investigating carbon partitioning over soybean seed development that can lead towards generating developmental flux maps. Understanding resource partitioning in developing seeds and identifying sources of carbon for undesirable oligosaccharide accumulation is key to reprogramming metabolism that yield enhanced oil and protein levels by decreasing carbohydrates.

Chemical jolt to increase storage lipid in microalgae Concetta C. DiRusso*, and Nishikant Wase, *University of Nebraska-Lincoln, USA*

Severe stress including nutrient deprivation leads to abundant accumulation of storage triglycerides in lipid droplets in algae. However, the same inducing conditions limit biomass and therefore ultimate yield. This has constrained the use of algae to produce biofuel lipids in an economically feasible manner. A high throughput screen of 44,000 small synthetic chemicals was performed and compounds were selected that increased lipid yield over time during growth. The compounds sorted both by phenotype and chemical structure to give 5

classes of unique lipid inducers. The compounds were extensively characterized and sorted by determining impacts on growth, TAG and/or starch accumulation, photosynthesis, respiration, induction of redox stress pathways and cellular metabolites. Two compounds, each of which increase lipids but vary in the impact on stress response, starch and metabolite profiles were further subjected to transcriptomic, proteomic and metabolomic analyses. The outcome is a metabolic signature that defines unique and common pathway determinants for algal cells treated with either compound. This database of information helps to define the metabolic rearrangement/reprogramming essential to induce lipid storage in algae. The compounds are expected to assist the successful employment of microalgae as feedstocks for biofuels and other bioproducts.

CoverCress – a Novel Oilseed Winter Crop with Canola-like Composition that Helps to Prevent Soil Erosion Tim Ulmasov*,*CoverCress, USA*

There is an urgent need for the development of oilseed crops that are suitable for both human and animal consumption and biofuel production, but which do not compete for land area with food crops. Pennycress (*Thlaspi arvense*), a member of the Brassicaceae, can be used to produce vegetable oil with attractive fatty acids profile that can also be used as a feedstock for biodiesel or jet fuel, as well as protein-rich seed meal that serves as animal feed. Pennycress seeds have high oil content (30-32%) with unique characteristics, such as superior cold-flow properties resulting from the lowest saturated fat content among commercially available plant-based oils (

Repurposing Carbon in Plant Leaves for Enhanced Agricultural Productivity Doug K. Allen*[1], Kevin Chu[2], Lauren Jenkins[3], and Shrikaar Kambhampati[4],*[1]Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA; [2]Donald Danforth Plant Science Center, USA; [3]USDA-ARS, United States; [4]Donald Danforth Plant Science Center, United States*

Improving plant yield through metabolic engineering is an important goal to meet future food and other renewable resource demands. At the heart of plant productivity is the balance of carbon assimilation through photosynthetic activity and its partitioning including steps that respire carbon as well those that produce protein, oil, carbohydrates or other compounds. The underlying metabolism that dictates which compounds and the relative amounts that are made can change over the course of development. In this talk I will present recent work with isotopic tracers and metabolite measurements that describe the dynamics of central carbon metabolism that result in altered balance between starch, protein, and lipid production in plant tissues. These studies are focused on enhancing lipid levels for biofuel needs and make use of methods we have recently developed to quantitatively assess acyl-acyl carrier protein levels that are the intermediates in fatty acid biosynthesis. Our studies indicate that in leaves engineered to make high levels of lipids, there is a tradeoff with accumulation of less non-transient starch. Acyl-ACP pools are significantly elevated possibly indicating that steps in lipid metabolism remain a bottleneck for further lipid increases. Current experiments and data will be presented that are aimed at further elucidating biochemical network operation and plant function.

Developing Healthier Oils and Other Food Ingredients through Genome Editing Javier Gil Humanes*,*Calyxt, Inc, USA*

Soybean oil has historically been partially hydrogenated to enhance its oxidative stability in order to increase shelf life and improve frying characteristics. This process, however, creates trans-unsaturated fatty acids, or trans fats. The discovery that dietary trans fats increase the risk of several health issues led the FDA to ban the use of partially hydrogenated oils, in processed foods, by all food manufacturers from June 18, 2018. Using transcriptional activator-like effector nucleases (TALEN®) technology, Calyxt developed a soybean trait that has produced oils with a fatty acid profile that contains approximately 80% oleic acid, 20% less saturated fatty acids compared to commodity soybean oil, and zero transfats. TALEN® technology is a precise genome editing tool that creates targeted cleavage of specific chromosomal sequences leading to knockout mutations at specific loci. To date, other crop species, such as wheat, potato and canola, have been edited at Calyxt to produce healthier food and food ingredients.

USDA Approach to Regulating Plant Breeding Innovation Neil E. Hoffman*, *USDA/APHIS, USA*

The Federal government has a coordinated, risk-based system to ensure that new biotechnology products are safe for the environment and human and animal health. Established as a formal policy in 1986, the Coordinated Framework for Regulation of Biotechnology describes the Federal system for evaluating products developed using modern biotechnology. The Coordinated Framework is based upon existing laws designed to protect public health and the environment. The U.S. government has written regulations, policies, and guidance to apply these laws to biotechnology-derived products. The U.S. Government agencies responsible for oversight of the products of agricultural modern biotechnology are the USDA's Animal and Plant Health Inspection Service (USDA-APHIS), the U.S. Environmental Protection Agency (EPA), and the Department of Health and Human Services' Food and Drug Administration (FDA). Depending on its characteristics, a product may be subject to the jurisdiction of one or more of these agencies. Regulatory officials from the three agencies regularly communicate and exchange information to ensure that any safety or regulatory issues that may arise are appropriately resolved. USDA first issued biotech regulations based on its statutory authority in 1986 and has only made modest revisions since. Major revisions were proposed in 2008 and 2017, however APHIS withdrew both rules in response to public comments and to reengage in a fresh dialogue with stakeholders on the regulation of biotechnology. Work is underway for a third proposed rule that optimistically will be published sometime in 2019.

Development of Strategies for Modification of Seed Oil Formation Randall J. Weselake*, *Department of Agricultural, Food and Nutritional Science/University of Alberta, Canada*

Vegetable oils are critical for food, feed and industrial applications, and in recent years global production of vegetable oil has been close to global demand. Triacylglycerol (TAG) is the predominant component of seed oil. In plants which produce seed TAG containing polyunsaturated fatty acids, TAG biosynthesis involves a complex interplay between the glycerol-3-phosphate pathway leading to TAG and membrane lipid metabolism. Metabolic engineering strategies aimed at increasing seed oil content and/or altering the fatty acid composition of seed oil have targeted one or more enzymes catalyzing key reactions in TAG biosynthesis. This presentation will include an

overview of our research directed towards increasing seed oil content in canola-type *Brassica napus* and gaining insight into the properties of type-1 diacylglycerol acyltransferase which catalyzes the final reaction in the acyl-CoA-dependent biosynthesis of seed oil. Possible mechanisms contributing to α-linolenic acid (ALA) enrichment of flax (*Linum usitatissimum*) seed oil will also be discussed. This basic information may be potentially useful for further increasing the ALA content of flax seed oil and the oils of other omega-3 fatty acid-producing organisms.

BIO 3.1/IOP 3/PRO 3.1: Biofuels

Chairs: Frank Dumeignil, Lille University, France; Xiaofei P. Ye, University of Tennessee, USA; and Megan Hums, USDA, ARS, ERRC, USA

Synthesis of Thiophene and Thiolane Derivatives Found in Biodiesel Produced from Brown Grease Lipids. Shehu Isah*, *Delaware State University-USDA, USA*

Brown grease lipids (BGL), the primary component of dewatered grease trap waste (GTW) and sewage scum grease (SSG) is a potential low-value feedstock for biodiesel production. Market limitations of these feedstocks for use in biodiesel production include high sulfur (S) content. A combination of analytical techniques including GC-FID, GC-PFPD, and GC-MS have been previously used to elucidate the identity of thiophene derivatives (C4H4S-X), thiolane derivatives (C4H8S-X) and other S-bearing compounds in BGL-derived biodiesel. These compounds do not exist in the MS library; therefore, a small degree of uncertainly surrounds their identification. These molecules cannot be isolated from biodiesel because their concentrations are too low. Therefore, this project was designed to synthesize the S-bearing compounds believed to be found in BGL-derived biodiesel in quantities sufficient to characterize them by analytical methods such as NMR. We have developed strategies to synthesize thiophene and thiolane and preliminary results indicate they can be produced in yields sufficient to assist in their characterization in biodiesel. The identification of S-bearing compounds in BGL-derived biodiesel is necessary to devise effective desulfurization protocols needed to reduce the concentration of S-bearing impurities in biodiesel to < 15 ppm, as specified by ASTM.

Modulating the Solubility of Saturated Monoglycerides (SMG) and Glycerol (GLY) in Blended Biodiesel Fuels Richard W. Heiden*[1], and Martin Mittelbach[2],[1]*R.W. Heiden Associates, LLC, USA;* [2]*Institute of Chemistry, University of Graz, Austria*

The unexpected in-situ formation of heterophases from residual impurities in biodiesel fuels has deleterious consequences stemming from limited solubility. To get beyond simple expressions of “like dissolves like” requires an understanding of compositional factors which promote or discourage entry of an impurity into the molecular network of the liquid fuel. As such, the liquid composition acts to modulate dissolution and precipitation processes. Diesel fuels are comprised of hundreds to thousands of distinct chemical compounds when mixed together in commercial blends of diesel #2(ULSD)diesel #1(ULS kerosene), renewable diesel (RD) and biodiesel FAME. Together with FAME these compounds create an environment with a defined polarity- a predisposition to modulate solubility that is determined by concentrations of main and minor hydrocarbon components at levels greater than about 0.1%. Despite the complexity in the molecular composition of biodiesel fuels, various international definitions and restrictions greatly narrow the range of possible compositions. However, low concentrations of impurities have intrinsic solubilities complicated by specific interactions*. We present here a study of possible relationships between the saturation points (SP) of GLY and SMG, fuel composition, and classical markers of solvent polarity, using existing theories to assist in explaining experimentally determined SPs and interactions. The results help establish an improved understanding of the compositional

factors defining solubility, the barriers imposed by current fuel definitions, and the magnitude of compositional changes needed to reduce the unwanted effects of impurities.
*Heiden,Schober, Mittelbach,(2017)JAOCS 94:285-299.

Co-production of acrylic acid in a typical biodiesel plant: a techno-economic assessment Xiaofei P. Ye*, *University of Tennessee, USA*

Producing value-added chemicals from glycerol is imperative for a sustainable future of biodiesel. Despite efforts worldwide, the commercial production of acrylic acid from glycerol faces challenges, both technologically and economically. Based on our patented technology using carbon dioxide as reaction medium in a two-step process to catalytically convert glycerol to acrylic acid, we established computer simulation models to analyze the energy efficiency and economics of the process. The analysis was conducted in conjunction with published data of a typical intermediate-sized biodiesel facility, aiming at the possibility of producing acrylic acid on site. Sensitivity analysis in response to the market value of glycerol, the source and cost of carbon dioxide recycling, and the changes in process kinetics will also be presented.

The Use of Controlled Flow Cavitation to Improve the Performance of Degumming, Refining and Biodiesel Operations Darren J. Litle*, *Arisdyne Systems, Inc., USA*

An overview of the application of controlled flow cavitation and compression-decompression jet atomization phenomenon for the intensification of chemical processing applications is presented. For vegetable oil acid degumming and/or neutralization reactions, the reasons for enhanced performance of the refining operation, reduced environmental impact, observed reduction in necessary acid and/or caustic addition as well as decrease in oil loss, potential savings in steam consumption and decrease in maintenance opex is discussed and industrial scale examples given. The efficient removal of residual soaps, phosphorus, ffa and metals while minimizing and in some cases even eliminating the need for water washing or silica addition is also described. Finally, the power of controlled flow cavitation to reduce catalyst consumption, increase throughput, and reduce monoglyceride content in finished biodiesel is also described.

BIO 4: General Biotechnology: Novel Lipids and Proteins

Chairs: Long Zou, Bunge Oils, USA; and Zheng Guo, Aarhus University, Denmark

"Directed Evolution and New-to-Nature Chemistry" - Experience of Working Together the Nobel Prize Laureate in Chemistry 2018

Zheng Guo*,*Aarhus University, Denmark*

The Nobel Prize in Chemistry 2018 was awarded with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies." Having been working in Frances Arnold´s lab for 1 year, In this talk, I will introduce latest and most exciting progress in enzyme technology area in the world leading lab in Caltech, Prof. Frances H Arnold's research philosophy and ideas, Particularly in "Learning from Nature" to "Teaching nature unnatural"; and how New-to-Nature Chemistry is created at the interface of enzyme technology and organic chemistry. Iron-catalyst based Haber–Bosch process to convert molecular nitrogen into ammonia for fertilizer, has arguably changed the world, which actually supports the Earth´s ever-growing population (least extra 3 billion people benefit for survival from this N-fixing technique). Once again Fe (Ru, Rh etc), by teaming up with protein scaffold, is shaking chemistry community, surprisingly bringing new-to-nature chemistry to life; where metalloenzyme carbenoids driven insertion reactions demonstrated how can enzymes do an amazing chemistry (forge C-C, C-S, C-Si, C-N and C-B etc bonds). For decades the enzyme technology for synthetic biotransformation does not really progress (swimming around hydrolases), particularly in underpinning organic synthesis that chemical catalysts created, until recently FH Arnold´s lab demonstrated engineered P450 is able to perform New-to-Nature chemistry; which initiate a flourishing interest after their seminal contribution.

Computational Protein Design Walter Rakitsky[1], and Alexandre L. Zanghellini*[2],*[1]TerraVia, USA; [2]Arzeda, USA*

The ability to use computational techniques to help design and improve nature's most powerful catalysts – enzymes – has been prophesized since the 70s. Fueled by the unprecedented biological data accumulation and increase in computational power over the last two decades, tools for the computational aided design of enzymes and proteins have advanced enough to enable us to solve some of the most difficult enzyme engineering challenges. This talk will highlight how Arzeda's protein design platform has demonstrated its efficacy in solving these challenges: from the engineering of truly novel catalytic function, to large active site reconfiguration for drastic substrate specificity and selectivity switches, and finally to the rapid generation of variant libraries for enzyme improvement. In the first part of our talk we will review Arzeda's published academic successes in the computational de novo design of synthetic enzymes with entirely new catalytic sites, with a special emphasis on the differentiating features of computational protein design. In the second part of the talk, we will present a success story of the application of computational enzyme design to solve an important industrial problem and creating value for one of Arzeda's partner. Finally, we will discuss the future of computational enzyme design and the tremendous impact it can have on industrial biotechnology.

Glycol-Functionalized Ionic Liquids for Enzyme Stabilization Hua Zhao*[1], and Gary A. Baker[2], *[1]University of Northern Colorado, USA; [2]University of Missouri-Columbia, USA*

This study aims to synthesize new glycol-functionalized ionic liquids with improved

enzyme activity and stability in non-aqueous environments. Glycol chains were grafted to various cations including imidazolium, pyridinium, ammonium, phosphonium, and sulfonium, etc. The physiochemical properties (such as viscosities and thermal stability) were measured for these functionalized ionic solvents. At first, we screened the lipase activities in these ionic media through a standard transesterification reaction, and then evaluated these ionic liquids in several enzymatic applications (i.e. enzymatic ring-opening polymerization, transesterification of triglycerides, and Michael addition). Our results suggest that the enzyme activity is highly dependent on the structures and properties of ionic liquids. Glycol-functionalization is an effective tool for tailoring ionic liquids with low viscosity and high enzyme compatibility.

Development of Novel Enzymes for Trans Fat-Free Oil Conversion Using Recurrent Neural Network-based Structure Prediction Andres E. Castillo*[1], Richard B. Rubin[2], Juan C. Duarte[1], and Leonardo Alvarez[1], *[1]Protera Biosciences, Chile; [2]Protera Biosciences, USA*

Recurrent neural networks (RNN) are applied to the challenge of protein structure prediction and enzyme functionality in our present work. We report on the application of this platform to develop new enzymes for highly selective triacylglycerol conversion to create trans fat-free oils with desired texture profiles. RNNs are artificial intelligence algorithms implemented recently in on-line translators significantly improving (often comical) sentences into coherent, context-specific language. We adapted RNN methodology to the prediction of 3D polypeptide structures from short amino acid sequences. The ability to employ highly parallel GPU computing with RNN results in accelerated machine learning allowing evaluation of 10^6 structures/minute. Faster *in-silico* prediction cycles enable the program to rapidly model accurately folded structures conforming to target triacylglycerols to obtain high performing enzyme candidates. Protein structure prediction programs built on prior generation computational methods typically require a feedback loop with extensive high throughput screening (HTS) assays of lab-generated proteins. By contrast, the RNN-based method developed aims to reduce viable protein candidates to a small number and generates scale-up ready biomolecules within just a few cost-effective computation:lab-test cycles. We are currently developing novel enzymes to perform biochemical saturation, which in contrast to chemical hydrogenation of fatty acids, doesn't generate trans fats in partially hydrogenated oils. After *in-silico* structure prediction, we insert the corresponding genetic sequences into *Pichia pastoris* to express enzyme candidates and then evaluate the novel activity on edible oils. Progress toward development of commercializable oils with target texture and melting point profiles through this enzymatic process will be presented.

The Next Generation of Immobilized Lipases for Interesterification in Vegetable Oil Processing Per Munk Nielsen, and Hans Christian Holm*, *Novozymes A/S, Denmark*

Around 200 oils and fats processing plants are using enzyme technologies to improve product qualities, process yield and production processes. The use of immobilized lipase for production of hard stocks for margarine has been on the market for more than 15 years. The enzymatic interesterification process is the state-of-the-art technology for production of larger production batches. The enzyme is formulated using a hydrophilic carrier material, the enzyme process ensures high processing yield, superior product quality and enable production without generation of trans fatty

acids. A new immobilized lipase offers further improvement for column bases interesterification process. In the past there has been a limit to the enzymatic interesterification process, as this has been cost efficient for large batches in continuous production setup. With the development of a new enzyme formulation and a new process for use of immobilized lipases it will be possible enter a new era of oil processing where enzymes are able to replace chemicals in batch processes. The new technology delivers increased production flexibility while still delivering a robust process with improved product quality and improved process economics.

Enhancing the Bioaccumulation of Curcumin in Caenorhabditis elegans by Using Nanoemulsion-based Delivery Systems Ruojie Zhang*[1], Peiyi Shen[2], D. Julian J. McClements[1], and Yeonhwa Park[3], *[1]University of Massachusetts Amherst, USA; [2]University of Massachusetts, USA; [3]Dept. of Food Science, University of Massachusetts Amherst, USA*

Curcumin is the principal curcuminoid of turmeric, which exhibits a broad range of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic capacities. Recently, there has been increasing interest in establishing the bioactivity of curcumin using Caenorhabditis elegans models since the genome of the nematode Caenorhabditis elegans shares many similarities with that of humans. In this study, a nanoemulsion-based delivery system was developed to deliver curcumin to C. elegans to increase its bioaccumulation and evaluate the body-fat reduction effects of curcumin. We found that Curcumin bioaccumulation increased with increasing droplet size of nanoemulsions, was higher for nanoemulsions containing corn oil than those containing fish oil or MCT, and was higher for droplets coated by whey protein than by Tween 80. The nematodes treated with curcumin-loaded nanoemulsions showed significantly reduced fat accumulation compared to the control group. This study could provide useful information to widen the application of C. elegans in research involving lipophilic compounds.

Biotechnology Approaches to Convert Sugars into Alka(e)nes: a Review Jingbo Li*, *MIT, USA*

Biofuels have been recognized as alternative liquid fuels to petroleum-based transportation fuels due to their combustion properties, renewable and sustainable advantages, and reduced climatic impact. Therefore, tremendous works focusing on bioethanol, biodiesel, and biobutanol production have been carried out. However, the energy density of ethanol and biodiesel is not as high as petroleum. In addition, these biofuels cannot completely replace petroleum. Alkenes and alkanes, composed of only C-H bonds, are a new generation of biofuels among the drop-in biofuels category. Recently, emerging biotechnologies have been able to convert sugars into drop-in fuels with certain yield. In this review, the metabolic pathways for converting sugars into fatty acids, the strategies to over-produce free fatty acids and medium chain length fatty acids, and the state of the art of converting fatty acids into alkanes/alkenes in vivo and in vitro are presented.

A New Stable Protease for Medical Instrument Cleaning Arjan Siebum*[1], Marvanne DeClerck[2], Jenny Newton[2], and Arjen J. Hoekstra[1],*[1]DuPont Industrial Biosciences, The Netherlands; [2]DuPont Industrial Biosciences, USA*

Cleaning is the critical first step when reprocessing surgical instruments prior to disinfection or sterilization. In many cases, the detergent used for this application contains enzymes such as protease to enhance cleaning efficacy. Protease enzymes contribute to the breakdown of proteins present in dried blood

which comprise a large portion of soils encountered in the medical equipment cleaning field. Enzymes such as amylase or lipase are often added to boost cleaning performance. The stability of enzymatic liquid detergent presents a challenge to formulators. A detergent used for medical instrument cleaning should typically maintain efficacy for up to two years. Enzymes are complex three dimensional proteins and are susceptible to breakdown by protease. Historically, there has been a limited number of approaches to stabilize protease enzymes: 1) adding stabilizers to the formulation, or 2) using a pre-stabilized protease. DuPont has used protein engineering techniques to develop a new stable protease for liquid detergents, which has proven to be particularly suitable for medical instrument cleaning formulations. Depending on the conditions a protease will lose activity upon dilution of the detergent, for example when carrying out manual soaking of medical devices. Soaking time could vary from a couple of minutes to several hours, and soaking temperature could vary from room temperature to typically 45 °C. An advantage of our structurally stable protease is its ability to maintain high residual catalytic activity under the aforementioned conditions for manual soaking and/or cleaning in an aqueous solution. This provides a more robust enzymatic solution for manufacturers of detergents for medical instrument cleaning, and therefore an opportunity for brand differentiation.

Solvent-Free Enzymatic Synthesis of Glyceryl Monogallate Optimized by Taguchi Method Siyu Zhang*, and Casimir C. Akoh, *University of Georgia, USA*

Gallic acid (GA) and its lipophilic forms, alkyl gallates, have been widely used in multi-industry fields as antioxidants. However, the potential harmful effects of alkyl gallates, such as estrogenic effects, limit their application and cause safety concerns. Glyceryl monogallate (GMG), on the other hand, has not been reported to have any adverse health effect. The enzymatic synthesis of many phenolic glyceryl esters has been achieved by *Candida antarctica* lipase B (CALB) recently. Due to the steric and electron-donating effects of GA, lipase-catalyzed conversion of GA has not been successfully achieved. In this work, GMG has been successfully synthesized for the first time by the enzymatic transesterification of glycerol and propyl gallate (PG). GMG was synthesized using an immobilized commercially-available food-grade enzyme, Lipozyme® 435, in solventless condition under atmospheric pressure with nitrogen flow. The effects of reaction variables (reaction time, temperature, enzyme load, and substrate ratio) on the enzymatic transesterification were studied and optimized by Taguchi method and regression analysis. The product was isolated and characterized by UV, FTIR, and electrospray ionization (ESI) high-resolution accurate-mass (HRAM) MS/MS. HPLC-DAD was used to monitor the reaction. Results showed that, GMG can be successfully prepared by the enzymatic transesterification of PG with glycerol in 65.6±1.0% yield at optimal conditions. The optimal conditions for this reaction were enzyme load 23.8%, PG: glycerol = 1:25 (mol/mol), 50°C, and 120 h.

Valorization of Fatty Alcohols: Derivatization of Fatty Alcohols into Novel Antioxidant Emulsifiers for Fish Oil Delivery Sampson Anankanbil*[1], Bianca Perez[2], Weiwei Cheng[3], Gustavo Gouveia, and Zheng Guo[4],[1]*Aarhus University, Department of Engineering, Denmark;* [2]*Dept. of Engineering, Aarhus University, Denmark;* [3]*South China University of Technology, China;* [4]*Aarhus University, Denmark*

Fatty alcohols are valuable chemical building blocks found in forestry wastes, wood pulp, natural oils and fats. The industrial

applications of fatty alcohols is limited by their poor reactivity, limited solubility in water-miscible solvents, high viscosity and high lipohilicity. The current work mitigated the above challenges by application of ring-opening reactions of phenolic anhydrides with lipophilic fatty alcohols to generate a new group of antioxidant amphiphiles (G8–G18), in a green and sustainable manner. Structural verification was by mass spectroscopy (MS) and nuclear magnetic resonance (1H/13C NMR) spectroscopy. Physicochemical characterization was by use of differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) spectroscopy, determinations of critical micelle concentrations (CMC) and hydrophilic-lipophilic (HLB) numbers. Antioxidant capacity was assessed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and hydroxyl radical scavenging activities. Dynamic light scattering (DLS) studies demonstrated surface-activity of G8–G18. Thiobarbituric reactive substances (TBARS) assay was used to monitor evolution of lipid oxidation in a model delivery system. Interfacial engineering of oil droplets in emulsions improved oxidative stability. Iron accelerated lipid oxidation at 22°C was significantly inhibited (up to 3.5 times) in emulsions stabilized by G8–G18 compared to emulsions stabilized by commercial emulsifiers and stabilizers. Thermal oxidation (at 80°C) was 10 times less in emulsions facilitated by G8–G18 compared to control emulsions stabilized by commercial emulsifiers and stabilizers. In conclusion, this work not only generated a new catalogue of antioxidant amphiphiles but also provides a feasible and scalable synthetic route for valorizing naturally occurring fatty alcohols, which are otherwise underutilized.

Physicochemical Properties of Oleogels Synthesized by Lipase-Catalyzed Interesterification Heejin Kim*[1], Nakyung Choi[2], No Young Kim[3], Hong-Sik Hwang[4], Byung Hee Kim[5], and In-Hwan Kim[6],*[1]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea; [2]Korea University, South Korea; [3]Korea university, South Korea; [4]USDA, ARS, NCAUR, USA; [5]Sookmyung Women's University, Korea; [6]Korea University, Republic of Korea*

A novel oleogel as a shortening replacer was produced via Lipozyme TL IM-catalyzed interesterification from rice bran wax (RBW) and high oleic sunflower oil (HOSO). The interesterification was carried out in a batch reactor at 70oC, 5% enzyme loading (based total substrate weight) with different blending ratio of RBW and HOSO (3%, 5%, and 7% RBW based on the total substrate weight). RBW, composed only of saturated fatty acids, is a wax ester consisting of 46 to 60 carbon. Composition of modified wax esters synthesized by interesterification was determined by gas chromatography with standard wax esters. To identify newly formed wax esters by interesterification, standards wax esters were prepared by Novozym 435-catalyzed esterification from authentic fatty acids and fatty alcohols. The novel oleogels prepared by interesterification were evaluated with various physical properties such as slip melting point, crystallization behaviors using differential scanning calorimetry, and microstructures using polarized microscope. The suitability of the oleogel as a shortening replacer was also studied by formulating several batters and baking them into cakes.

BIO 4.1 / S&D 4: Biosurfactants and Environmentally Friendly Ingredients
Chairs: Sujan Singh, Arkema, USA; and Douglas Hayes, University of Tennessee, USA

Comparative Antimicrobial Efficiency Among C18 and C22 Sophorolipid Congeners towards Select Gram+ Bacterial Strains Richard D. Ashby*, and Daniel K.Y Solaiman, *USDA, ARS, ERRC, USA*

Many microbial glycolipids are effective antimicrobial agents. Sophorolipids (SLs) are naturally composed of sophorose (a disaccharide) connected to a fatty acid 'tail' through a glycosidic bond and depending on the producing strain, the substrate, and the culture conditions, the structure of the lipid tail may vary in terms of chain length and number of olefinic groups. The antimicrobial activity of SL seems to be greater against Gram-positive (Gram+) bacteria. This presentation will focus on the antimicrobial efficiency of different SL congeners against select Gram+ bacterial strains including *Propionibacterium acnes*, various *Streptococci* and *Lactobacilli* strains commonly found in the oral cavity and *Listeria monocytogenes*, a strain associated with food-borne illness. This presentation will present our recent findings on the production and purification of C22 SL isoforms from *Pseudohyphozyma bogoriensis* and provide a comparative assessment of the antimicrobial efficiency for various SL congeners produced by both *Starmerella bombicola* and *P. bogoriensis*.

How Biosurfactants Can Enable Degreasing J. R. Bennett[1], Eric Theiner*[1], and Stephanie Hackney[2],[1]*Evonik Corporation, USA;* [2]*Evonik Corporation, United States*

Biosurfactants such as sophorolipids and rhamnolipids are gaining considerable interest because of their utility in forming emulsions as well as the potentially positive toxicity and environmental attributes. By combining these types of surfactants with environmentally preferable lipophilic materials one can take advantage of basic emulsion properties to show effective degreasing. This presentation will show the benefits of one approach in removing heavy industrial greases and the resulting formula options revealed.

The Combined Effects of Soap and Sophorolipids in the Development of Mild Body Wash for Sensitive Skin Glen Lelyn Quan*[1], Chie Matsubara[1], Yoshihiko Hirata[1], Satoshi Yoshida[1], Maiko Iwai[1], Shinji Hamaguchi[1], Etsuko Komiyama[2], and Shigaku Ikeda[1],[1]*Saraya Co., Ltd. , Japan;* [2]*Juntendo University, Japan*

Skin care is one of the cited pillars of treatment according to the Atopic Dermatitis Treatment Guidelines released by the Japanese Ministry of Health, Labor and Welfare. In order to control *Staphylococcus aureus* flora, the skin should be washed by showering, bathing, followed by topical treatment as necessary. However, routine washing can be harsh to the skin, making milder cleansing agents more desired. Sophorolipids are promising glycolipid biosurfactants which have known characteristics such as excellent detergency and good rinsability, to name a few. Another quality to explore is its low-irritating effect on the skin, making them suitable to be used in formulations for sensitive skin. We tried to incorporate sophorolipids in formulations with soap and other components to develop a mild body wash. After establishing a stable formulation, effects of sophorolipids in the formulation were observed through sensory tests and their effect on the stratum corneum barrier function, followed by safety evaluation and comparison with other commercial products. Furthermore, a clinical evaluation involving 200 patients of Juntendo University Hospital with various skin diseases (including

those with atopic dermatitis) was also performed. The obtained data showed improvement on the degree of skin dryness and skin itchiness of patients after two weeks of use. It can be concluded that the combined effect of soap and sophorolipids in the developed mild body wash provide cleansing action that is also safe and soothing, even for patients with sensitive skin.

Optimal Regulation of Oxygenation for Coordination of Rhamnolipid Productivities and Residual Fatty Acid Content in Fermentation of *Pseudomonas aeruginosa* Qin Meng*,*Zhejiang University, China*

Rhamnolipids are multipurpose surface-active molecules produced by the bacterium Pseudomonas aeruginosa. Despite the high reputation of biosurfactants, such as low toxicity, biodegradability and high stability, these compounds have not been widely used because of the high cost of production and the difficulty on purification. Vegetable oil is believed to be the best raw material for rhamnolipid fermentation. The rhamnolipid synthesis from oil prefer to a medium aeration, which provide an inhibition of the microaerobic denitrification. Nevertheless, the severe foaming become a big problem in aerobic rhamnolipid fermentation, decreasing the rhamnolipid yield. Meanwhile, the residual fatty acid with similar lipid tails as rhamnolipids usually exacerbate the difficulties on the final separation and purification which are critical for the bioprocess of biosurfactant detergent. Hence, dissolved oxygen concentration (DO) will be extremely important in coordination of foaming, denitrification and β-oxidation in biosynthesis of rhamnolipids by fermentation. This study will monitor the rhamnolipid productivity and residual fatty acid content using different oxygenation level which was represented by the oxygen transfer coefficient (KLa) as a result of agitation and aeration.

Aspartic Acid-based Ampholytic Amphiphiles: Synthesis, Characterization, and pH-Dependent Properties at Air/Water and Oil/Water Interface Weiwei Cheng[1], Sampson Anankanbil[2], Liu Guoqin[3], and Zheng Guo*[4],*[1]South China University of Technology, China; [2]Dept. of Engineering, Aarhus University, Denmark; [3]School of Food Science and Engineering, South China University of Technology, China; [4]Aarhus University, Denmark*

A facile and two-step strategy was employed to synthesize two series of novel aspartic acid-based ampholytic amphiphiles from sustainable and commercially viable substances as starting materials. The molecular structures of the synthetic compounds were well identified by MS and 1H/13C analysis, and the physicochemical, pH-dependent foaming and emulsifying properties were evaluated by the use of multiple techniques such as FTIR, DSC, Langmuir−Blodgett study, and fluorescence microscopy imaging. Due to the coexistence of amino and carboxyl groups in the synthetic compounds, the compounds presented varying charges (cationic, ampholytic, and anionic) depending on the pH of the medium compared to the dissociation constants (pKa). Compounds with cationic (pH 1.0) and anionic (pH 9.0) forms had significantly higher γ0.1 and CMC values than that with ampholytic forms (pH 7.0). Sn-1-lauroyl-sn-3-aspartic acid (compound 3) at neutral and alkaline conditions displayed comparable foaming properties including foaming, calcium-tolerance, and temperature-resistance ability with commercial sulfonate SDS, and thus might be a promising alternative to SDS, applied in personal care products and detergent formula. Sn-1-palmtoyl-sn-3-aspartic acid (Compound 5a) with ampholytic structure was proved as the most excellent stabilizer for the preparation of oil-in-water nanoemulsions compared with palmityl aspartic acid (compound 5b), commercial food ingredient DATEM, and

glyceride monopalmitate at aqueous phase pH 7.0. Thus, it has promising use as a pH-dependence emulsifying agent in various fields.

Antimicrobial Efficacy of Oxygen-Based Bleach Systems Sam Adamy*, *Church & Dwight Co. Inc., USA*

Systems which generate oxidative molecules in the form of a peroxide or a peracid are attractive for use in a number of applications, not only from the standpoint of stain removal, but for antimicrobial action as well. Such systems additionally exhibit favorable environmental and toxicity profiles, thus making them well-positioned for consumer and regulatory acceptance. For example, two components associated with oxygen-based bleaching, sodium percarbonate and tetraacetylethylenediamine (TAED), have been safely employed in the marketplace for many years, and produce relatively benign waste streams. Achievement of required efficacies for sanitization and/or disinfection claims can be challenging, however. In this presentation, examples of oxygen-based bleaching systems and associated efficacies in laundry and other home care applications will be presented, along with comments around the applicability to claims of disinfection and sanitization. Data will be presented on both antibacterial and antiviral outcomes.

Fatty Acid, Methyl Ester and Vegetable Oil Ethoxylates George A. Smith*, *Sasol North America, USA*

Soap is the first and oldest example of a biobased surfactant. Soap is prepared by saponifying fats and oils with caustic. Soap has been used for over 4500 years for personal cleaning and washing of clothes. In personal cleaning products, the high pH of soap can irritate the skin and eyes and soap also suffers from sensitivity to salinity and hard water ions. With the advent of modern industrial chemistry, the reliance on soap has declined. Fatty acid ethoxylates (FAE) are prepared by reacting fatty acids with ethylene oxide or polyethylene glycol (PEG). Both reactions produce a mixture of ethoxylated fatty acid, bis fatty acid ester and PEG. FAE can be used in neutral pH and is relatively insensitive to hard water ions but suffers from low yields due to the transesterification reaction. Methyl ester ethoxylates (MEE) were originally developed to improve the efficiency of fatty acid ethoxylates. Reacting fatty acid methyl ester with ethylene oxide using a calcium or magnesium based catalyst or esterification of fatty acids with polyethylene glycol methyl ether (MPEG) produces MEE high yeilds. MEE is soluble at neutral pH and shows favorable detergency but suffers from hydrolytic stability issue in alkaline solution and does not build viscosity in low active formulations through the salt effect. Vegetable oil ethoxylates (VOE) are made by direct ethoxylation of triglycerides or transesterficiation of tryglycerides with ethoxylate glycerin. VOE show good surface activity and are exceptionally mild to skin and eyes. VOE is used primarily in personal care as emollients and foam boosters in rinse off products.

Biobased Surfactants: An Overview Douglas G. Hayes*, *University of Tennessee, USA*

Biobased surfactants continue to gain increased attention and employment, despite the relatively low cost of fossil fuels in today's world. In this paper, I will provide a review of biobased surfactants, providing information on underlying trends relating to biobased surfactants, particularly in terms of environmental sustainability, and describe new and emerging biobased surfactants.

Laundry Sustainability vs. Laundry Sanitization: The Tension and the Solutions Nancy Falk*, *Clorox, USA*

Most of the laundry process carbon footprint is related to the energy required to heat the wash water. As a result, wash temperatures have decreased, chemistries have become less harsh, and cloth-to-wash water ratios have increased. Studies have also shown that microbial contamination in laundry is common and spreads readily within and between washloads, exposing consumers to pathogens. In this talk, innovations in laundry to advance sustainability are contrasted with microbial contamination, and current chemical solutions are reviewed.

Biodegradable Dispersants for Phosphate Free Automatic Dishwashing Detergents Scott A. Backer*[1], Severine S. Ferrieux[2], Eric P. Wasserman[1], Paul P. Mercando[1], Randara Pulukkody[1], Anurima Singh[1], Lin Wang, Ken Laughlin[4], Steve Arturo[1], and Lu Bai[1], *[1]Dow Chemical Company, USA; [2]The Dow Chemical Company, France*

Over the last decade, a significant shift in the sustainability profile for detergent formulations has been taking place. In order to combat eutrophication of waterways, regulations requiring the removal of sodium tripolyphosphate from detergents in the developing world have been proposed and implemented. This has radically altered the strategies of formulators, as new combinations of ingredients are required to take the place of once-abundant phosphates. One area of research has been on novel polymers capable of dispersing inorganic salts formed as detergents come into contact with hard water. These dispersants, classically low to moderate molecular weight polyacrylic acids, are excellent performers which demonstrate only minimal biodegradability. This talk will discuss strategies used to design and test a new class of biodegradable dispersants which exceed the performance of current dispersants while significantly increasing the overall level of biodegradable polymeric carbon.

Greener and Milder Functionalized Sugar-Based Surfactants for Home Care and Industrial Applications Robert J. Coots*, Dennis Abbeduto, and Andy Sun, *Colonial Chemical, USA*

Colonial Chemical Inc. is a leading supplier of naturally-derived, functionalized sugar-based surfactants with the trade names of Suga® and Poly Suga®. These products meet the growing demands in Household and Personal Care industries to replace ingredients which have Prop 65 concerns or are highly irritative, such as alcohol alkoxylates and sulfates. Suga®Nate is a series of 100% naturally-derived anionic polyglucoside surfactants, produced using concepts well known in the field of green chemistry, as opposed to the traditional means for manufacturing sulfates and sulfonates. These ingredients are qualified for Safer Choice Direct Release and they show no, or very low irritation to eyes and skin. These surfactants show good detergency and foaming and have unique attributes to applications in home, pet, and vehicle care applications. Suga®Fax D10NC is a 100% natural, green hydrotrope with improved performance versus Sodium Xylene Sulfonate (SXS). This product can be used at lower levels with improved performance while avoiding the toxic impurities in SXS, which are of Prop 65 concern. Poly Suga®Mulse products represent 100% biobased, EO-free emulsifiers for fragrances and other emulsion formulations. The nonionic surfactants function much like ethoxylated fatty alcohols, with superior performance and without the concern of residual EO or 1,4-Dioxane. Poly Suga®Quat surfactants, with their cationic nature, have been shown to boost cleaning of greasy soils, and have the ability to boost the efficacy of a preserved formulation.

BIO-P: Biotechnology Poster Session

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

1. Absolute Quantification of Acyl-ACPs by Mass Spectrometry Lauren M. Jenkins*[1], Bradley S. Evans[2], and Doug K. Allen[3],[1]*USDA/Donald Danforth Plant Science Center, USA;* [2]*Donald Danforth Plant Science Center, USA;* [3]*Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA*

Acyl carrier proteins (ACPs) are an important scaffold for fatty acid biosynthesis. Measuring their levels can help to understand regulation of lipid biosynthesis in plants. The elongating fatty acids are attached to a serine residue of ACP within the conserved amino acid sequence (Asp-Ser-Leu-Asp; DSLD) through a 4'-phosphopantetheine arm. Recently developed methods take advantage of this sequence and digest with aspartate-N endoproteinase (asp-N) to produce a chain covalently attached to a three-amino acid sequence (acyl-DSL) which is detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The objective of this work is to synthesize an isotopically labeled standard for absolute quantification of acyl-ACPs in oilseeds. Sfp synthase (4'-phophopantetheinyl transferase; Sfp) was used to enzymatically transfer the acyl group from acyl-CoA to the conserved serine side chain on an apo-ACP. Then the acyl-ACPs were digested with asp-N preceding LC-MS/MS detection. Results indicate that Sfp is efficient in transferring medium chain acyl groups to E. coli ACP (C6 – C10) and less so in transferring long chain acyl groups (C14 – C18) due to the insolubility of long chain CoA's in the presence of the high levels of Mg2+ required for Sfp transferase activity; however, nonionic detergents solubilize long chain acyl groups effectively.

2. Carbon Partitioning in Chlamydomonas reinhardtii under Autotrophic and Mixotrophic Conditions for Growth and Biomass Production Kevin P. Foley*[1], Zoee Perrine[2], James Umen[2], and Doug K. Allen[3],[1]*Donald Danforth Plant Science Center, USA;* [2]*Donald Danforth Plant Science Center, USA;* [3]*Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA*

Photosynthetic cells including algae are a critical component of the earth's carbon cycle and have biotechnological relevance as potential bioenergy feedstocks and producers of high value compounds such as nutritional supplements. Inorganic carbon in the form of carbon dioxide is taken up by photosynthetic cells and converted into sugars and amino acids that are the building blocks used to produce more cells (i.e. growth), or alternatively that can be used to produce starch and lipids which serve as storage reserves. Very little is known about the metabolic control mechanisms that direct carbon towards these different fates. This project uses the single celled green algal reference organism Chlamydomonas to investigate carbon partitioning under different growth conditions that include autotrophic or mixotrophic metabolism. We will describe the differences in biomass production and pathway use as determined by stable isotope labeling and metabolic flux analysis. These studies will provide a deeper understanding of a key aspect of photosynthetic metabolism and enable the development of strategies for manipulating algae or other photosynthetic cells to improve yields of biotechnologically relevant compounds.

3. Modeling Pulse/Pulse-Chase Radiolabeling to Assess Lipid Metabolism Doug K. Allen*[1], and Philip D. Bates[2],[1]*Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA;* [2]*Washington State University, USA*

Lipid flux has historically been considered through radioisotopes (14C, 3H). Transient labeling reflects the precursor-product relationships; however distinguishing models based on the inspection of labeling curves is difficult and not always intuitive given complexity of lipid metabolic networks (below). A computational model was built to consider differences in network structure involved in active lipid metabolism. Ordinary differential equations were used to describe pulse and pulse-chase labeling experiments based on mass action kinetics. Models may inform/explain lipid engineering strategies.

4. Characterization of Central Carbon Metabolism in High Oil Tobacco Lines Over Development Kevin L. Chu*[1], Lauren Jenkins[2], Sally K. Bailey[3], Shrikaar Kambhampati[3], Philip D. Bates[4], and Doug K. Allen[5],[1]*Donald Danforth Plant Science Center, USA;* [2]*USDA-ARS, USA;* [3]*Donald Danforth Plant Science Center, USA;* [4]*Washington State University, USA;* [5]*Agricultural Research Service, U.S. Department of Agriculture / Donald Danforth Plant Science Center, USA*

Plants can provide a renewable source of triacylglycerols (TAG) for biofuel and chemical feedstock applications. Though plants normally only accumulate significant amounts of lipids in seed/fruit tissue, engineering leaf tissue to accumulate TAG could increase oil yields due to decreased energetic costs of sugar translocation as well as increased capitalization of the plant life cycle for vegetative biomass production. In *Nicotiana tabacum*, the combined overexpression of multiple genes involved in different aspects of TAG synthesis and stabilization has led to the accumulation of over 30% TAG in leaves (DW). To probe the metabolic consequences of forcing leaves to become dual source-sink tissues, we seek to perform isotopically nonstationary metabolic flux analysis. We therefore characterized leaves from both wild-type and high-oil lines across development by quantifying metabolites of interest while also measuring 13C-label incorporation from $^{13}CO_2$-labeling studies. A redirection of carbon from starch to lipid synthesis in high-oil leaves is suggested by the absence of accumulation of non-transitory starch observed in WT leaves over development. These studies thus provide insight into the metabolism of merged source-sink leaves over development while also optimizing tobacco leaf labeling.

5. Chemical Composition of a Human Milk Fat Substitute Produced by Enzymatic Interesterification Roberta Claro da Silva[1], Rafaela Airoldi*[2], Juliana N.R Ract[3], Iván Jachmanián[4], Heather L. Colleran[5], Salam A. Ibrahim, and Luiz A. Gioielli[3],[1]*North Carolina A&T University, United States;* [2]*Sao Paulo University, Brazil;* [3]*University of Sao Paulo, Brazil;* [4]*UdelaR, Uruguay;* [5]*North Carolina A&T State University, United States*

The study and development of new human milk fat substitutes (HMFS) have significant economic and industrial importance since the production of HMFS has relevance in public health in cases where breastfeeding is not possible. The objective of this study was to produce structured lipids HMFS (lard - LA and coconut oil- CO) added with polyunsaturated fatty acids (Single Cell Oils - SCO). The individual oils and four different blends (A-50% CO + 50% LA, B - 50% OC + 50% SCO, C -50% LA + 50% SCO and D – 33% CO + 33% LA + 33% SCO) were interesterified using Lipozyme RM IM as catalysator. The oils and blends were analyzed by fatty acid composition (FA), triacylglycerol

(TAG) composition and regiospecific distribution. The FA composition of the pure lipids showed a promising source of FA to produce HMFS. The interesterification of blend A increased TAGs with 38, 40 and 42 ECN. The blend B showed an increase in the percentages of TAGs with 32, 38 and 42 ECN and also presented a new TAG with 40 ECN. The blend C showed 33 peaks of triacylglycerols with a broad distribution of triacylglycerols with TAGs from 32 to 52 ECN. The main triacylglycerols present are those with 44, 46 and 48 ECN groups, which are present in the three oils studied. The blend C after interesterification showed 73.9% of the saturated fatty acids esterified at sn-2 position, while unsaturated fatty acids preferentially occupied the sn-1,3 positions, as in human milk fat.

6. Human Milk Fat Substitute Produced by Continuous Enzymatic Interesterification: Effect of Different Reaction Parameters

Roberta Claro da Silva[1], Heather L. Colleran[2], Juliana N.R Ract[3], Salam A. Ibrahim, Luiz A. Gioielli[3], and Ezinne C. Chukwu*[2],[1]*North Carolina A&T University, USA;* [2]*North Carolina A&T State University, USA;* [3]*University of Sao Paulo, Brazil*

The modification of fats and oils for infant formulas in order to obtain not only the similar fatty acid composition but also the same positional distribution as in human milk fat substitute (HMFS) via interesterification needs to be intensely investigated. The objective of this study was to evaluate the effects of temperature (50°C and 70°C), flow speed (1,2,3,4 and 6 mL/min) and catalysator (Lipozyme RM IM and TL IM) during continuous enzymatic reaction of a mixture containing 85% lard + 10% coconut oil + 5% single cell oils to produce HMFS. The lipases were subjected to the conditioning process until the acid level was reached below 2g oleic acid/100g sample. The reactions were performed in a tubular glass bioreactor provided with an external jacket for constant temperature and with a fixed bed to support the lipase (5g). The obtained structured lipids were analyzed for regiospecific distribution by nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC) and crystallization under polarized light microscopy (PLM). Continuous enzymatic interesterification was effective with the two lipases used in all temperatures and flow conditions. The interesterification showed very similar results for the two lipases. Lipozyme RM IM was more efficient at 50°C in higher flows (3 and 4 mL/min) and the reaction using Lipozyme TL IM showed no difference between the temperatures. The thermal behavior confirmed the effectiveness of the reaction in all conditions, as well as the similarity between the structured lipids produced.

7. Specialized Lysophosphatidic Acid Acyltransferases Contribute to Unusual Fatty Acid Accumulation in Exotic Euphorbiaceae Seed Oils

Jay Shockey*, *SRRC-ARS-USDA, USA*

Many exotic Euphorbiaceae species, including tung tree (Vernicia fordii), castor bean (Ricinus communis), accumulate unusual fatty acids in their seed oils, many of which are valuable industrial feedstocks. However, production of these fatty acids in transgenic plants often results in various adverse plant characteristics including low seed yields, production of toxic compounds, limited growth range, and poor resistance to abiotic stresses, thus limiting full agronomic exploitation of these plants. Biotechnological production of these unusual fatty acids (UFA) in high yielding non-food oil crops would provide new robust sources for these valuable bio-chemicals. Previous research has shown that multiple oil metabolism genes must be included to drive efficient selective UFA incorporation into seed lipids. Here we use demonstrate that lysophosphatidic acid acyltransferases from two Euphorbiaceae species have high selectivity for

incorporation of their respective unusual fatty acids into the phosphatidic acid intermediate of oil biosynthesis. These results are consistent with the hypothesis that unusual fatty acid accumulation arose in part via co-evolution of multiple oil biosynthesis and assembly enzymes that cooperate to enhance selective fatty acid incorporation into seed oils over that of the common fatty acids found in membrane lipids.

8. Enhancing acetyl-TAG synthesis through metabolic engineering of the oilseed crop *Camelina sativa*. Timothy P. Durrett*[1], Linah Alkotami[2], and Catherine Kornacki[2],[1]*Kansas State University, USA;* [2]*Kansas State University, United States*

Many *Euonymus* species produce unusual structured triacylglycerol (TAG) molecules referred at as acetyl-TAG, because they possess an *sn*-3 acetate group instead of a long chain fatty acid present in regular TAG. The high viscosity and melting point of vegetable oil, mainly consisting of regular TAGs, prevent its direct use as biofuel for diesel engines. Acetyl-TAGs however bypass such drawbacks due to reduced viscosity and superior cold temperature properties, facilitating potential use as improved diesel 'drop-in' replacements. To produce large quantities of acetyl-TAG-rich oil, *Euonymus alatus* diacylglycerol acetyltransferase (*EaDAcT*) was previously expressed in the oilseed crop *Camelina sativa*, resulting in up to 65 mol% of acetyl-TAG in the seeds. Expression of *Euonymus fortunei* diacylglycerol acetyltransferase (*EfDAcT*), shown to possess higher in vitro acetyltransferase activity compared to EaDAcT, increased acetyl-TAG accumulation to 85 mol%. Suppression of the endogenous TAG competing enzyme (DGAT1) further enhanced acetyl-TAG accumulation to 90 mol% in selected transgenic lines. Accumulation of high levels of acetyl-TAG demonstrated little or no impact on seed size, weight, and fatty acid content. High acetyl-TAG containing seed exhibited a two-day delay in germination compared to wild-type seed. Quantification of EfDAcT protein levels in developing seed revealed that in high acetyl-TAG producing lines, EfDAcT protein expression is not limiting acetyl-TAG accumulation. The results provide new insights on factors limiting acetyl-TAG accumulation.

9. Modification of Alkyl Chain Length Composition of Microalgae Nannochloropsis Tatsurou Ozaki, Shinji Sugihara*, Mayumi Wada, Akihito Kawahara, Takeshi Saito, and Yasushi Takimura,*Kao corporation, Japan*

Various length of fatty acids are utilized as wide range of industrial raw materials. Nannochlorpsis, which is high oil contents marine algae, produces mainly C16 and C18 Fatty acids and a acertain level of C20 including eicosapentaenoic acid (EPA), but few amount of medium chain length fatty acids. In this study, to obtain several types of fatty acid from a strain Nannochloropsis, we attempted to develop the genetic modification technologies for fatty acid composition control of Nannochloropsis based on cisgenesis technology (self-cloned). Candidate genes involving alkyl chain length control in the lipid metabolism are cloned from Nanochloropsis cDNA library and overexpressed in the cell of Nannocloropsis. Medium chain fatty acids were dramatically increased by overexpression of Thioesterase and Ketoacyl synthase obtained from Nannochlrospsis cDNA library. Fatty acid composition of the modified strain reached maximum 50% of medium chain fatty acids (including C10 to C14). Moreover, we discovered the effective genes involving long chain fatty acid production from Nannochloropsis genome and achieved to obtain modified Nannochloropsis strain which enriched palmitoleic acid or eicosapentaenoic acids by cisgenesis technologies. We discuss future utilization of the technology to produce

several fatty acids as industrial raw material based on Carbon Capture and Utilization aspect.

10. Modeling and Optimization of Lipase-Catalyzed Hydrolysis of Phosphatidylcholine Using Response Surface Methodology for L-α-Glycerylphosphorylcholine Production Yejin Song*[1], Soo Jeong Lee[1], Jung Eun Lee[2], Eunji Choi[2], and Byung Hee Kim[3],*[1]Sookmyung Women's University, South Korea; [2]Sookmyung Women's University, Republic of Korea; [3]Sookmyung Women's University, Korea*

Modeling the lipase-catalyzed hydrolysis of soy phosphatidylcholine (PC) in a biphasic medium for the production of L-α-Glycerylphosphorylcholine (L-α-GPC) and optimizing the reaction conditions using response surface methodology were described. The reaction was performed with 4 g of PC in a stirred batch reactor using a lipase as the biocatalyst. The effects of temperature, reaction time, water content, and enzyme loading on L-α-GPC content in the reaction products were elucidated using the models established. Optimal reaction conditions for maximizing the L-α-GPC content were as follows: temperature, 55 °C; reaction time, 4.9 h; water content, 105.9% of the PC weight; and enzyme loading, 9.4% of the PC weight. Under these conditions, PC was completely hydrolyzed into L-α-GPC.

11. Characterizing Monosaccharides and Starches in a Co-Culture of Microalgae Chelsea M. Tyus*, Zhimin Xu, Maria Teresa Gutierrez-Wing, JeeWon Koh, and Joan King,*Louisiana State University, United States*

Microalgae is a source of functional ingredients with positive health effects due to PUFAs, polysaccharides, pigments, essential minerals, vitamins, enzymes and bioactive peptides. This study focused on *Chlorella vulgaris* (Chlorophyta) /*Leptolyngbya sp.* (Cyanobacteria) co-culture microalgae (CCA) carbohydrate characterization, data that is needed for application of these carbohydrates in the future. CCA obtained from the LSU Aquatic Resources Engineering was lyophilized prior to all assays. Total monosaccharide content (TMC) was analyzed using the Phenol Sulfuric method. Total Starch (TS) was quantified using Total Starch HK Kit. An Amylose/Amylopectin Kit provided CCA starch characteristics. A Resistant Starch Kit provided the amount of resistant and non-resistant starch present in CCA. GC-MS was used to identify monosaccharides present in CCA. TMC was calculated as 20-25% of CCA this coincides with previous studies (Kent et. al 2015; Kumar et. al 2016). TS was determined as 31.32g/100g of algae DWB. Amylose content was 31.92%; amylopectin content was 68.08% determined by subtraction. Resistant starch content was 0.50g/100g of algae DWB; non-resistant starch was 18.93g/100g of algae DWB. D(+) Glucose (1079.67 µg/mg), D(+) Galactose (94.00 µg/mg), and D(+) Mannose (69.84 µg/mg) were identified and quantified by GC-MS proximate quantification with standards. Microalgal products need to become more diversified and economically competitive. The consumer demand for healthy, plant-based, sustainable, high-protein foods is increasing. CCA carbohydrates could be used as a source of plant-sourced carbohydrates and starches in foods. They also display rheological attributes when sulfated and could then be used as foaming-agents, stabilizers, and emulsifiers in food.

12. Solvent Fractionation Method for Preparing Pinolenic Acid Concentrates from Pine Nut Oil Fatty Acids Min-Yu Chung[1], In-Hwan Kim[2], and Byung Hee Kim*[3],*[1]Korea Food Research Institute, South Korea; [2]Korea University, Republic of Korea; [3]Sookmyung*

Women's University, Korea

The aim of this study was to prepare pinolenic acid (PLA) from free fatty acids (FFA) obtained from pine nut oil using solvent fractionation. Siberian pine nut oil containing 18.3 wt% PLA was used as the starting material for the fractionation. The fractionation was performed in *n*-hexane at ultra-low temperatures down to −85°C. The PLA concentrates produced under the optimal conditions established in this study (temperature, −85°C; *n*-hexane- to-FFA ratio (v/w), 30:1; fractionation time, 36 h) contained 69.8 wt% PLA. The yield of PLA was 77.4 wt% of the initial PLA weight in the FFA. These results suggest that solvent fractionation is a more effective approach to prepare PLA concentrates with higher PLA contents at a particular yield of PLA than published methods using urea crystallization (e.g., PLA content = ~47 wt%, yield of PLA = ~77 wt%, Woo et al. (2016)) or lipase-catalyzed reactions (e.g., PLA content = ~30 wt%, yield of PLA = ~61 wt%, Lee et al. (2011)). The resulting PLA concentrates contained 11 of the 12 different species of FA present in the FFA, thereby indicating that the PLA concentrates prepared by solvent fractionation have more diverse FA profiles than those prepared by urea crystallization (e.g., 7 species of FA, Woo et al. (2016)).

13. Preparation of Oleogel from Sunflower Wax and a Vegetable Oil in a Packed Bed Reactor via Lipase-Catalyzed Interesterification

Jihyun Kim*[1], Nakyung Choi[1], Heejin Kim[2], Hong-Sik Hwang[3], Byung Hee Kim[4], Phyrim Lee[5], and In-Hwan Kim[6],[1]*Korea University, South Korea;* [2]*Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea;* [3]*USDA, ARS, NCAUR, USA;* [4]*Sookmyung Women's University, Korea;* [5]*Department of Integrated Biomedical and Life Science, Graduate School, Korea University, South Korea;* [6]*Korea University, Republic of Korea*

The purpose of this study was to prepare a novel oleogel in a packed bed reactor via lipase-catalyzed interesterification from sunflower wax and high oleic sunflower oil. Lipozyme 435 from *Candida antarctica* was employed as a biocatalyst. Composition of wax esters after interesterification were determined by gas chromatography using standards wax esters. The standard wax esters were prepared by enzyme-catalyzed esterification from standard fatty acids and standard fatty alcohols. The novel oleogel prepared by interesterification was evaluated with various physical properties such as slip melting point, crystallization behaviors using differential scanning calorimetry, solid fat content, and microstructures using polarized microscope. The novel oleogel as a shortening replacer was applied for the preparation of cake.

14. Optimize the Astaxanthin Production Platform by Using Optima Cultural Condition Analysis of Chlorella. sp. DT and the Transgenic Approach

Wei-Tin Lin[1], Yi-Ting Kou[2], Cheng-Huan Liu[2], Wei-Ting Lin[2], and Yu-Ting Chen*[2],[1]*Institute of Genomics and Bioinformatics, National Chung Hsing University, Taiwan;* [2]*Institute of Genomics and Bioinformatics, National Chung Hsing University, Taiwan*

Microalga enriched in multiple nutrition is wildly applied on functional food industry. Especially, the astaxanthin, a potent antioxidant, plays with anti-tumor, anti-oxidation, regulating immunity, lowering blood pressure and cholesterol, accelerating cell repair and promoting child growth and other physiological functions. Endemic to Taiwan, Chlorella. sp. DT was isolated from cable lines on mountain area. It can survive by 42°C dehydration and then rehydration. During this treatment the carotenoid was accumulated to protect the cell damage by dehydrated. We first discovered that the Chlorella. sp. DT produce

astaxanthin. In this study, for astaxanthin production, Plackett-Burman method was used to explore the most significant impacts of medium and related regulators in promoter region. Then, we used response surface methodology to predict the optimum medium composition for Chlorella. sp DT astaxanthin production. Further, we cloned βbeta-carotene ketolase, the key enzyme of astaxanthin production, and transfer the gene by Agrobacterium into tobacco to catalyze the astaxanthin formation in chloroplast. Transgenic N. tabacum was yield 604.6 μg g-1 (dw), the highest astaxanthin production in nuclear transformation system. The construction of transgenic tobacco and astaxanthin production optimization platform of Chlorella. Sp. DT will improve the astaxanthin production and application in agriculture, medicine, food, industry and other related industries. We also established the semi-productive supercritical extraction system of astaxanthin, which yields 37% higher than the acetone extortion. User can take it as a low-cost and solvent-free residue extraction platform in the production of safe and functional ingredients.

15. Production of Natural Rubber by Lactuca spp. Tom McKeon*, *Agricultural Research Service, USA*

Natural rubber is an essential industrial product, but the sole source is the tropical tree *Hevea brasiliensis* which is highly susceptible to a fungal pathogen. As a result, there is great interest in developing alternative sources of natural rubber. These include *Parthenum argentatum* (guayule), *Taraxacum kok-saghyz*(Russian dandelion)and *Lactuca* species (edible and wild lettuces). We have been examining rubber and resin content of latex from Parris Island Cos lettuce during flowering, finding differing levels of rubber in latex from floral tissue versus bolting stem. Preliminary results indicate the molecular weight of rubber from both sources is >1 million daltons, similar to natural Hevea rubber. Evaluation of rubber content from young leaves of the wild lettuce *L. virosa*(opium lettuce) found minor amounts of rubber, with additional screening needed as the plant matures.

16. Polyol Obtained from Liquefaction of Nicotiana tabacum Stalks using PEG – Glycerin Blend Chiragkumar M. Patel*, *Industrial Chemistry Dept., 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 160ºC. The liquefied product showed an IOH of 200 to 400 mg KOH/g and a viscosity of 0.93 Pas. The developed polyols can be used for development of foams, adhesives, 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.

17. A New Methodology for the Process Monitoring of Enzymatic Proteolysis by Size-exclusion Chromatography Sophie Beaubier*[1], Irina Ioannou[1], Xavier Framboisier[2], Olivier Galet[3], and Romain Kapel[2],[1]*LRGP - UMR CNRS*

7274, France; [2]Reaction and Process Engineering Laboratory UMR-7274, France; [3]Avril Group, France

Enzymatic proteolysis is an industrial process used in a wide range of applications. Study of this process consists in kinetic follow-up of the protein conversion rate (Xp), the mean peptide size (Naa) and the hydrolysis degree (DH). The communication describes an original methodology to quantify simultaneously these three criteria by size-exclusion chromatography (SE-HPLC). Xp is simply deduced from the evolution of protein peak area (column dead volume) in the course of the reaction. For Naa and DH, the method converts peptide absorbances into concentrations by applying Beer-Lambert law. To do so, a molar extinction coefficient is assessed for each chromatogram point. The overall concentration signal is integrated and Naa is calculated with the ratio of the molar quantities of amino acids to peptides in the hydrolysate. DH is deduced from the ratio of Xp and Naa. At first, the approach was tested on the hydrolysis of bovine serum albumin, lysozyme and rapeseed albumin by Alcalase 2.4L. Values of DH were also determined by TNBS and pH-stat methods. Most of the hydrolysate obtained showed relative differences < 20% with the reference methods. Then, the method was adapted to fit the TNBS assay. 39 experimental validation tests were analyzed by SE-HPLC, TNBS and pH stat methods. 90% of the validation data show non-significant differences between the DH predicted and the DH measured by TNBS method. The proposed methodology can be efficient for the process monitoring of enzymatic proteolysis while minimizing time and quantity of sample assay required.

18. A Methodology to Predict Kinetics of Protein Enzymatic Hydrolysis Sophie Beaubier*[1], Claire Defaix[1], Xavier Framboisier[2], Olivier Galet[3], and Romain Kapel[2],[1]*LRGP - UMR CNRS 7274, France; [2]Reaction and Process Engineering Laboratory UMR-7274, France; [3]Avril Group, France*

Enzymatic proteolysis has been used in a wide range of applications. This process is an effective way to improve protein nutritional and functional properties. Proteolysis kinetics and performances depend on several operating conditions (pH, temperature (T), ratio Enzyme/Substrate (E/S)). To date, there is a lack of rationality in proteolysis process implementation. This is mainly due to the reaction complexity that involves multiple substrates with possible changing exposure. Several approaches were proposed to model proteolysis kinetics. At the best, these approaches were able to predict degree of hydrolysis (DH) kinetics as a function of T and protein initial concentration or E/S. This work proposes a new methodology to simulate both DH and protein conversion rate (Xp) kinetics as a function of 3 operating conditions (T, pH and E/S). To appropriately describe the system, three assumptions were made. Xp and DH kinetics follow 2nd order reaction models. Maximum hydrolysis terms (Xpmax and DHmax) only depend on the pH of hydrolysis. The other kinetics terms depend on the three operating conditions. In the proposed methodology, Xpmax and DHmax are determined by preliminary experiments. Correlation models (pH, T and E/S vs Xp and DH kinetic terms) were obtained by non-linear regressions. The proposed methodology was applied to the rapeseed albumin hydrolysis simulation with Alcalase 2.4L. DoE methodology identified the correlation between operating conditions and kinetic terms. The ANOVA showed that the 2 models were reliable (R^2 for k(Xp)= 0.95; for k(DH)= 0.85; p-value <0.05). Furthermore, no significant lack of fit was observed.