106th AOCS Annual Meeting and Industry Showcases

Biotechnology Division Technical Program Abstracts

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

BIO 1: Biocatalysis I

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

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

Characterization of *Brassica napus* Diacylglycerol Acyltransferase 1 and the Enzyme's N-terminal Region. K.M.P. Caldo, M.S. Greer, G. Chen, M.J. Lemieux, and R.J. Weselake*, University of Alberta, Canada.

Diacylglycerol acyltransferase (DGAT) is a membrane-bound enzyme that catalyzes the acyl-CoA-dependent acylation of *sn*-1,2-diacylglycerol to form triacylglycerol. Highly purified recombinant *Brassica napus* DGAT1 (BnaC.DGAT1.a) formed dimers and tetramers in n-dodecyl-ß-D-maltopyranoside micelles. The predominant dimeric form was purified about 126-fold over the solubilized fraction and exhibited substrate preference for α-linolenoyl-CoA > oleoyl-CoA > palmitoyl-CoA > linoleoyl-CoA > stearoyl-CoA. The recombinant soluble N-terminal region corresponding to BnaC.DGAT1.$a_{1\text{-}113}$ was produced in *Escherichia coli*, purified and found to form tetramers. Truncation of this domain showed that the dimeric interface is located within amino acid residues 49 to 113, while the first 48 amino acid residues may be involved in tetramer formation. Circular dichroism (CD) analysis showed that BnaC.DGAT1.$a_{1\text{-}113}$ is mainly α-helical in a membrane-mimetic environment. Binding analysis using Lipidex-1000 previously demonstrated that the N-terminal fragment can interact with acyl-CoA through positive cooperativity suggesting that this region represented an allosteric exosite. In the current study, ligand perturbation analysis monitored using CD also showed that oleoyl-CoA could interact with this possible regulatory domain.

Transgenic Oil Palm: Evaluation of Various Methods, Target Tissue, and Selection Agents. G.K.A. Parveez, M.Y.A. Masani, A.M.D. Izawati, O.A. Rasid, B. Bahariah, M.S. Masura, A.N. Hanin, W.S.W.N. Syuhada, A.R. Nurfahisza, and I.N. Fakhrana, Malaysian Palm Oil Board, Malaysia.

Oil palm industry is faced with many challenges and keeping the industry remains competitive is essential. One of the efforts is to utilize modern biotechnology or genetic engineering to improve value of this crop. Among the targets for genetic engineering of oil palm are increasing oleic acid, ricinoleic acid, palmitoleic acid and stearic acid content, enhancing lycopene content and synthesizing biodegradable plastics. Genetic engineering requires a transformation method to be developed to transfer gene of interest into oil palm. Transgenic oil palm was successfully developed in 1997 using biolistics approach. Since then a number of transformation methods were explored and successful production of transgenic oil pam mediated by *Agrobacterium* was achieved. Optimization of the transformation methods together with evaluation of various target tissues and selection agents were later initiated and are ongoing. Latest development and challenges faced during the research and potential solution will be elaborated.

Optimum Conditions for the Production of Soy Polyol Oils and Diacylglycerol from Soybean Oil by *Acinetobacter haemolyticus* A01-35 NRRL B-59985. C.T. Hou and K. Ray, NCAUR, USDA, USA.

Triacylglycerols (TAG) containing hydroxy fatty acids has many industrial uses such as the manufacture of aviation lubricant, plastic, paint, nylons and cosmetics, because of the hydroxyl groups on the fatty acid (FA) constituents. Diacylglycerols (DAG) containing hydroxy FA can also be used in the above mentioned industries. Soy-polyols (oxygenated TAG) are important starting materials for the manufacture of polymers such as polyurethane. Recently we developed a new method to screen microorganisms for the direct production of polyol oils from soybean oil. One of the cultures, Acinetobacter haemolyticus A01-35 NRRL B-59985, produced DAGs containing hydroxyl FA and DAGs with normal FA from soybean oil (BAB 2: 1-6, 2013). Now we determined the optimum conditions for the production of these products as: pH 6.8; temperature 28°C; time 3 day. With galactose as carbon source it produced more polyol oils whereas with mannose it produced more DAG with normal FA. Yeast extract at 10g/L plus trypton at 5-7g/L serves as better nitrogen sources. The culture medium is best without NiCl2, CoCl2, or nicotinic acid. With optimum conditions, culture A01-35 produced 4.42mg polyol oils and 23.31mg DAG with normal FA per 30ML culture medium.

Gut Microbial Polyunsaturated Fatty Acid Saturation Metabolism Generating Bio-active Hydroxy, Oxo, and Conjugated Fatty Acid Derivatives. J. Ogawa[1], S. Kishino[1], T. Sugawara[1], S. Tanabe[2], and T. Kawada[1], [1]Kyoto University, Japan, [2]Hiroshima University, Japan.

PUFAs derived from dietary lipids were found to be saturated by gut microbes through biohydrogenation metabolism. We revealed the metabolism in representative gut bacteria, lactic acid bacteria. The enzyme system was found to consist of four enzymes. The concerned action of these enzymes, i.e., hydratase, dehydrogenase, isomerase, and enone reductase accomplish the saturation and generated hydroxy fatty acids, oxo fatty acids, and conjugated fatty acids as intermediates. We confirmed the existence of these fatty acids in host tissues depending on the existence of gut microbes and evaluated their physiological activity. 10-Hydroxy-cis-12-octadecenoic acid, an initial metabolite of linoleic acid, contributed to recovery from damage to the intestinal epithelial barrier. Furthermore, hydroxy fatty acids and oxo fatty acids controlled lipid metabolisms through interactions with nuclear receptors such as PPARs and LXR. These results indicated that these fatty acid molecules formed by gut microbes affect the health of the host. The hydratase catalyzing the initial step of the metabolism with FAD and NADH as cofactor and activator, respectively, was applied for the production of hydroxy fatty acids useful as bio-active lipids and materials for polymer synthesis.

Various Rare Polyunsaturated Fatty Acid Productions by *Mortierella alpina* Breeding. A. Ando[1], T. Okuda[1], H. Kikukawa[1], E. Sakuradani[2], J. Shima[3], J. Ogawa[1], and S. Shimizu[4], [1]Kyoto University, Japan, [2]University of Tokushima, Japan, [3]Ryukoku University, Japan, [4]Kyoto Gakuen University, Japan.

A filamentous fungus, *Mortierella alpina* 1S-4, belonging to the Zygomycetes, has been isolated from soil as a potent producer of polyunsaturated fatty acids (PUFAs) in our laboratory and used for commercial production of arachidonic acid (AA, 20:4n-6). A host-system for *M. alpina* 1S-4 was developed by means of molecular breeding for improving and modifying PUFAs productivity and composition. We developed a transformation system for this fungus to improve the fatty acid composition. In this study, we demonstrate various rare polyunsaturated fatty acid productions by oleaginous filamentous fungus *M. alpina* breeding.

Enzymatic Synthesis of Tyrosol-based Phenolipids: Characterization and Antioxidant Activities. G. Pande and C.C. Akoh*, University of Georgia, USA.

Oxidative stability of lipids is one of the most important parameters affecting their quality. Lipase-catalyzed lipophilic tyrosyl esters with equivalent carbon alkyl chain but different unsaturation (C18:0 to C18:4n3) were prepared, characterized, and used as antioxidants. Free fatty acids and their ethyl esters (substrate molar ratio tyrosol: acyl donor, 1:10) were used as acyl donors and immobilized lipase from *Candida antarctica* as biocatalyst (10%). The phenolipids were isolated and characterized using ESI-MS, ^{1}H NMR, and ^{13}C NMR. Peroxide value (PV) and *para* anisidine value (*p*AV) were measured to evaluate their antioxidant activities in bulk oil (structured lipid, SL) and in an oil-in-water emulsion (SL-based infant formula). No significant difference was found in yield and reaction time between the two types of acyl donors. However, as the unsaturation of the fatty acids increased the reaction time also increased. In SL, tyrosyl esters exhibited lower antioxidant activity than tyrosol whereas the addition of an alkyl chain enhanced the antioxidant efficiency of tyrosol in infant formula. Tyrosyl oleate was the most efficient antioxidant in the emulsion system followed by tyrosyl stearate and tyrosyl linoleate. These results suggest that the synthesized phenolipids may be used as potential antioxidants in lipid-based products.

Novel Marine Carotenoids and Their Functions. M. Hosokawa, N. Takatani, T. Sawabe, and K. Miyashita, Hokkaido University, Japan.

Carotenoids are naturally occurring yellow, orange, and red pigments. To date, more than 750 carotenoids have been isolated from natural sources. These carotenoids have a variety of biological functions in photosynthesis and photo-protection, in stabilizing membrane fluidity. Some kinds of carotenoids have attracted greater attention because of their beneficial effects in the prevention of serious diseases such as cancer and metabolic syndrome. We isolated novel monocyclic carotenoids, 2'-isopentenylsaproxanthin, 3"-hydroxy-2'-isopentenylsaporoxanthin, which are "Chimera"-like unique structures, in that one end group forms the same structure of zeaxanthin, and the other end group is the same as bacterioruberin, from *Jejuia pallidilutea* strain 11shimoA1 and *Gillisia limnaea* in the family *Flavobacteriaceae*.

Furthermore, 2-hydroxyflexixanthin was also isolated from marine bacteria, strain oki45. DPPH radical scavenging and singlet oxygen quenching activities of these monocyclic carotenoids were higher than that of β-carotene.

Selective Antibacterial Activity of Palmitoleic Acid Useful for Possible Prevention of Atopic Dermatitis. T. Nagao[1], S. Tanaka[1], A. Kurata[2], H. Nakano[1], and N. Kishimoto[2], [1]Osaka Municipal Technical Research Institute, Japan, [2]Kinki University, Japan.

Atopic dermatitis is an allergy occurred by several factors such as allergen, genetic factor, stress, and microorganisms. *Staphylococcus aureus* is not so much observed in healthy control skin, but *Staphylococcus epidermidis* is alternatively observed and shows *S. aureus* growth inhibition. Meanwhile, *S. aureus* dramatically increases in atopic dermatitis inflammation part, and is concerned in aggravation of the inflammation.

Palmitoleic acid (9-*cis*-C16:1) is observed in limited natural oils such as macadamia nut oil. This fatty acid is an isomer of sapienic acid (6-*cis*-C16:1) observed in human skin, and both C16:1 shows antibacterial activity against *S. aureus*. Since sapienic acid decreased in the atopic dermatitis skins and is rarely observed in natural oils, supplement of palmitoleic acid to the skin should be useful for repression of *S. aureus*. However, palmitoleic acid shows antibacterial activity against *S. epidermidis*. We thus aimed for investigation of several factors for selective antibacterial activity of palmitoleic acid: repression of *S. aureus* and no repression of *S. epidermidis*. The results showed that weak acid condition was an important factor, and oleic acid observed in many natural oils inhibited the antibacterial activity.

Production of Diacylglycerols by Glycerolysis of Soybean Oil Catalyzed by an Immobiled Lipase in a Bubble Column Reactor. Y. Wang, X. Yang, N. Zhang, and Y. Teng, Jinan University, China.

Preparation of Diacylglycerol (DAG) by Lipase-catalyzed glycerolysis of soybean oil and glycerol (GLY) was carried out in a bubble column reactor using Novozym435 under a solvent-free system and the effects of enzyme load (oil and GLY mass), mole ratio (GLY to oil), temperature, gas flow and reaction time were investigated. The optimal conditions of glycerolysis reaction were as follows: enzyme load 4wt%, molar ratio (GLY to oil) 20:1, temperature 90^{o}C, gas flow 0.83L/min and reaction time 150 min. Under the selected conditions, the diacylglycerol content reached 49.41 wt%. After purified by molecular distillation, the diacylglycerol content was achieved to 63.55%. And thermal property of diacylglycerol and soybean oil characterized by differential scanning calorimetry had obvious difference.

BIO 2: Biocatalysis II

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

Chairs: C.T. Hou, USDA, ARS, NCAUR, USA; and L.K. Ju, University of Akron, USA

Reforming of Sucrose Fatty Acid Esters by Lipase Reactions. Y. Nishiyama, T. Aibara, H. Uehara, and Y. Ueda, The Nisshin OilliO Group, Ltd., Japan.

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

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

Effects of Particle Size of Sucrose Suspensions and Pre-incubation of Enzymes on Lipase-catalyzed Synthesis of Sucrose Oleic Acid Esters. R. Ye[1], D.G. Hayes[1], and R.M. Burton[2], [1]University of Tennessee, USA, [2]MARC-IV Consulting, Inc., USA.

The effects of high-speed homogenization, high-intensity ultrasound, and their combination were evaluated for the reduction of the particle size of sucrose crystals to enhance solvent-free lipase-catalyzed synthesis of sucrose oleate at 65°C. The combination of homogenization and ultrasound greatly decreased the particle size of suspended sucrose crystals in mixtures of oleic acid/ sucrose oleate (86wt% monoester and 14wt% diester) at a ratio of 90/10 w/w from 88 to 18µm. The suspension-based medium was charged to a stirred tank bioreactor that also contained immobilized lipase from *Rhizomucor miehei* or *Candida antarctica* (Lipozyme®IM and Novozym® 435, respectively; Novozymes, Franklinton, NC, USA), that was pre-incubated in oleic acid for several different temperatures (23–60°C), durations (4–24h), and stir rates (50–400rpm, (radius of 3cm), prior to use. The optimal performance was achieved using *C. antarctica* lipase (83.3wt% ester, consisting of 46wt% monoester) in the presence of molecular sieves (18wt%). The low water concentration (~0.12wt%) did not affect the activity of *C. antarctica* lipase.

Economic Model for the Glucose/Oleic Acid-based Synthesis of Sophorolipids and Some Potential New Applications for These Glycolipid Surfactants. R.D. Ashby, D.K.Y. Solaiman, and L.S. Liu, USDA, ARS, ERRC, USA.

Sophorolipids (SLs) are microbial glycolipids (biosurfactants) that are produced by Candida yeasts and are continually finding new applications. It is generally acknowledged that SLs require higher production costs than petroleum-based surfactants but; no in-depth economic study has been made to prove this assumption. This presentation will focus on the development of a process economic model for the synthesis of SLs from glucose (Glc) and oleic acid (OA) using current production practices, contemporary process simulation software and current reagent, equipment, and supply costs but other considerations such as capital, labor, material, and utilities costs were also included. The greatest contributor to the overall production cost of SLs was determined to be the raw materials which accounted for 87% of the total estimated production expenditures. Assuming yields of 100g/L, the cost of large-scale (90.7 million kg/yr) synthesis was determined to be US$2.54/kg ($1.15/lb). Additionally, 2 new applications for SLs will be discussed including its use as an effective antimicrobial agent against the acne-causing bacterium Propionibacterium acnes and as an additive to thermoplastic polyhydroxyalkanoate (PHA) biopolymer films for material property control.

Genetically Engineered Rhamnolipid-producing Organism for Glycerol Utilization. D.K.Y. Solaiman and R.D. Ashby, USDA, ARS, ERRC, USA.

Rhamnolipid (RL) is currently developed for industrial use as a biobased surfactant that also has

antimicrobial activity attractive for applications in sanitizing washes. Glycerol stream from biodiesel production is a low-cost substrate for microbial fermentation. *Pseudomonas chlororaphis* is a non-pathogenic organism preferred for RL production, but it cannot metabolize glycerol. We report here the PCR-cloning and expression of *E. coli* glycerol facilitator (*glpF*) and glycerol kinase (*glpK*) in *P. chlororaphis* to overcome its shortcoming of glycerol utilization. These genes were spliced downstream from a *Pseudomonas* promoter (P2) in a pBS29-P2-gfp vector. The growth of *P. chlororaphis* transformants were compared using glycerol as a sole substrate. The results showed that concomitant expression of *glpF* and *glpK* caused faster cell growth than the wild-type and the transformants expressing only *glpF* or *glpK*. The final glycerol consumption and cell density, however, were similar in all tested organisms, indicating that *glpF* and *glpK* together govern the kinetics of the initial glycerol metabolism. HPLC-ELSD analysis showed that RL synthesis was not affected in transformants. The results lay the groundwork for further improvement of glycerol utilization in *P. chlororaphis* through metabolic engineering of the subsequent steps in the pathway.

Waste Oil/Grease Conversion and Biodiesel Feedstock Production by Phagotrophic Algae. C. Li, J. Kohl, S. Xiao, M. Hosseini, Z. Lin, N. Vongpanish, and L.K. Ju*, University of Akron, USA.

Phagotrophic algae can engulf small insoluble liquid and solid entities and metabolize these entities internally as food. This ability makes phagotrophic algae potentially good candidates for treating and/or converting waste oil/grease, organic solid waste, waste activated sludge from wastewater treatment plants, and various waste and byproducts from food and agricultural industries. Phagotrophic algae can perform phagotrophic, heterotrophic and phototrophic metabolism. We have shown that the selected species can accumulate high amounts of lipids, 30%-80% of cell dry weight, depending on conditions. We have studied the growth and lipid production from several types of waste. In one study, we investigated the feasibility of pretreating waste cooking oil (WCO) to lower free fatty acid content by using the algae. The algal cells engulfed oil droplets and grew rapidly on WCO samples. The algae-pretreated oil had lower acid values, by 2-3mg KOH/g WCO, and were then converted to biodiesel. Phagotrophic algae have so far been under-utilized for industrial purposes. Our work aims at developing a new phagotrophic algae-based platform of converting waste to useful lipids and oil.

An Innovative Technology for Synthesis of Biodiesel Using Defatted Rice Bran as a Biocatalyst. I.H. Kim and N. Choi, Korea University, Republic of Korea.

Numerous researches regarding rice bran lipase focused on the deactivation of its activity to stabilize the rice bran because the rice bran lipases possess high hydrolytic activity. However, little was done any attempt for the utilization of rice bran lipase. In this study, surprisingly, biodiesel was synthesized successfully from soybean oil and methanol using defatted rice bran as a biocatalyst. The 15wt% of soybean oil (based on the weight of rice bran) and a desired amount of methanol was wetted to the defatted rice bran. The effect of molar ratio (fatty acid to methanol), reaction temperature, and water content were investigated. The optimum conditions were selected for 1:2 of molar ratio, 40^{o}C of temperature, and 12wt% of water content. After the 12 days of reaction, the biodiesel yield of 90.4wt% was obtained under the optimum conditions. The relative activity of enzyme was investigated on the every 12 days of reaction under the optimum condition. The relative activity decreased gradually and was 83.5% of relative activity of enzyme was obtained after 7 cycles.

Applicability of a Novel Enzymatic Method to the Analysis of Positional FA Distribution in Milk Fat. Y. Watanabe[1], R. Hori[2], Y. Miyazaki[3], T. Nagai[4], K. Saito[5], T. Sano[2], A. Sasaki[6], R. Sasaki[7], S. Sato[8], C. Sato[6], T. Shibuya[9], Y. Tsukahara[9], A. Yamashita[10], K. Yoshinaga[4], S. Watanabe[11], [1]Osaka Municipal Technical Research Institute, Japan, [2]J-Oil Mills, Inc., Japan, [3]NOF Co., Japan, [4]Tsukishima Foods Industry Co., Japan, [5]Kao Co., Japan, [6]The Nisshin OilliO Group, Japan, [7]Miyoshi Oil & Fat Co., Japan, [8]Japan Food Research Laboratories, Japan, [9]Showa Sangyo Co., Japan, [10]ADEKA Co., Japan, [11]Fuji Oil Co., Japan.

Milk fat contains short chain fatty acids (SCFAs) which are considered to contribute to the easiness to digest for babies and to the milky odor. The positional distribution of FAs in milk fat was analyzed by a newly developed enzymatic method based on 1(3)-selective transesterification at 30^{o}C for 3h of target oil (0.5g) with ethanol (5.0g) by immobilized *Pseudozyma* (*Candida) antarctica* lipase (Chirazyme L-2, C4, Roche Diagnostics). The resulting 2-MAG was fractionated by Sep-Pak silica cartridge (0.65g, Waters), methylated and brought to GC analysis. Little problem was found in the enzymatic part of the protocol by the collaborative study organized by

Japan Oil Chemists' Society. However, RSDs of SCFA contents were relatively large in the GC analysis, probably due to the volatility and solubility to the aqueous phase of SCFAs. Propylation instead for methylation, and the selection of the capillary column for GC reduced RSDs to <15%, which was in an acceptable range for SCFAs of <3wt%. It is also proposed to determine SCFA contents by propylation and long chain FAs by methylation, and to combine the two results adjusting in the level of C12, using FID correction factors.

BIO 2.1/IOP 2/PRO 2: Alternative Fuels and Enzymatic Biodiesel

Chairs: H.C. Holm, Novozymes A/S, Denmark; R.M. Burton, Novozymes, USA; G. Knothe, USDA, ARS, NCAUR, USA; and S. Lewis, Solenis, USA

Development of Enzymatic Catalyzed Fat-splitting Processes. A. Rancke-Madsen, P.M. Nielsen, and H.C. Holm, Novozymes A/S, Denmark.

Conventional thermal fat-splitting processes suffer from expensive and complex equipment, high energy consumption, product quality problems like *trans* fats and color formation and hazardous work environment. Enzymatic catalyzed fat-splitting processes are attractive potential alternatives but enzymatic processes still only have very limited industrial uses, mainly due to insufficient temperature stability and relatively high cost of current enzymes like *Candida rugosa lipase.*

Thermostable lipases expressed in high yields by industrially relevant host systems have been tested at 55°C/131°F in 100ml shaking glass reactor system using a wide range of feed-stocks, including tallow and acid oil waste materials, 30% water w/w and 0.1-1.0% w/w enzyme solution.

The results suggest new lipases have been identified which are superior to traditional fat-splitting lipases, due to better thermostability, lower cost and high reaction rates. Kinetic studies and initial engineering assessments suggest enzymatic catalyzed fat-splitting technology will become successful in the industries within a few years.

Evaluation of Glycerol Carbonate Production and Its Cosynthesis in Enzymatic Biodiesel Production. R.M. Burton[1] and J. Greenstein[2], [1] MARC-IV Consulting, Inc., USA, [2]North Carolina State University, USA.

Glycerol is a byproduct of biodiesel production. In large centralized biodiesel production facilities, glycerol may be refined to a high purity product. Yet for many biodiesel plants crude biodiesel glycerin is still a waste concern. Glycerol (1,2,3-propane triol) is a trifunctional molecule that can be modified to produce a wide range of products. Glycerol carbonate (GC) and glycidol (2,3-epoxy-1-propanol) are key intermediates in the production of many of these products. GC is a “platform chemical” which can help substitute bio-based feedstocks for the current petroleum derived sources. Recently, researchers have begun investigating the use of enzymes to convert glycerol to glycerol carbonate. In addition, biodiesel production using enzymatic catalysis has also moved from the research phase to the commercial arena. When enzymatic catalysis is employed for biodiesel production, there is an opportunity to obtain a higher quality of glycerol co-product. This higher glycerol quality may allow an easier processing to new biobased materials. Here, we will evaluate both the enzymatic conversion of glycerol to glycerol carbonate as well as the enzymatic co-synthesis pathway of biodiesel and glycerol carbonate together.

New Developments in Enzymatic Catalyzed Biodiesel Improve the Process Significantly. P.M. Nielsen, A. Rancke-Madsen, T. Balle, B. Knuthsen, and H.C. Holm, Novozymes, Denmark.

The enzymatic catalyzed biodiesel has been in large scale production for more than two years most of the time in a test period in a few plants, but now it also available for all biodiesel producers. During that period a lot have been learned and the process has been improved.

The technical improvements in the process and lower enzyme costs open the possibility of using the enzyme for one batch only. This has some big impacts on how the process can be designed. In the presentation we will discuss the implications of one time use of liquid lipase including: the reaction can be operated at higher temperature and methanol concentrations leading to shorter reaction time and easier separation of heavy phase after the reaction. The downstream process for enzyme recovery can be omitted, and it opens for the possibility of continuous reaction system.

Continuous Enzymatic Biodiesel Processing. B. Chrabas, Viesel Fuel, LLC, USA.

Feedstock costs are recognized as the highest variable cost in the production of biodiesel accounting for up to 80% of the material costs associated with making a gallon of biodiesel. The Enzymatic Biodiesel Pathway allows for the use of feedstocks which are lower in cost by comparison and have limited use in conventional biodiesel processes.

The ability to optimize process inputs in a continuous system lead to the development of the Continuous Enzymatic Biodiesel Process. Working in collaboration with Novozyme, Tactical Fabrication, and Purolite, the Skunkworks Team is pioneering the

use of enzymes in their Continuous Enzymatic Biodiesel Process.

The Continuous Enzymatic Biodiesel Process being demonstrated by Viesel Skunk Works, LLC at its World Headquarters in Stuart, FL has been used as a test-bed for the scalable process.

The basic tools required for an enzymatic biodiesel laboratory and how they aid in determining the suitability of various lower cost feedstocks will be outlined in this presentation accompanied by production data taken from the Continuous Enzymatic Biodiesel Processor.

Cold Flow Properties of Fatty Acid Methyl Esters: Additives versus Diluents. R.O. Dunn, USDA, ARS, NCAUR, USA.

Biodiesel is typically composed of fatty acid methyl esters (FAME) converted from agricultural lipids. Common feedstocks include soybean, canola, rapeseed, sunflower and palm oils. Recent debate on the conversion of edible oils into non-food products has created opportunities to develop alternative non-edible feedstocks such as jatropha and used cooking oils, waste grease and animal fat. The cold flow properties of biodiesel are poor compared to conventional diesel fuel (petrodiesel). Vehicles fueled by biodiesel/petrodiesel blends may experience start-up and operability problems if exposed to overnight temperatures below the cloud point (CP). Performance issues are exacerbated when the biodiesel is made from high-saturated fatty acid feedstocks including palm oil and many of the aforementioned non-edible oils. Technical strategies have been devised to improve the cold flow properties of biodiesel. Although cold flow improver (CFI) additives can decrease pour point (PP) and cold flow plugging point (CFPP), these additives do not significantly improve the CP when employed at low concentrations (< 1%) in biodiesel and biodiesel/petrodiesel blends. However, increasing the concentration of some additives (diluents) was more effective. This report provides an updated perspective on the development of new CFI additives and diluents for biodiesel.

Fuel Quality Sensors for Characterization of Biofuels and Determination of Their Aging Degree. J. Krahl, M. Eskiner, and Z. Fan, Coburg University of Applied Sciences and Arts, Germany.

A fuel sensor should prevent engines from damages in the fuel line or the combustion chamber or the exhaust gas treatment system. Two sensor principles are introduced to control the quality and the age of biofuels: Dielectric relaxation and fluorescence spectroscopy.

The detection principle of the newly designed dielectric sensors for determining the aging degree is based on dielectric relaxation spectroscopy. The sensor is characterized by its simplicity, its small size and low price. A possible future use could be the installation in fuel tanks to control the oligomer concentration.

By measuring the real and imaginary part of permittivity in a broad frequency range, it is possible to observe relaxation processes, because of a lag in response of high-molecular and polar oxidation products from biodiesel in the alternating electric field.

Time resolved laser induced spectroscopy (TRLSF) allows identification and quantification of diesel fuel without sample preparation. Applying a mathematical principal component analysis allows the determination of FAME from different feedstocks in diesel fuel. In a further development step a miniaturization of fluorescence sensor was achieved. This LFS-prototype provides approximately similar features as the TRLFS, but without time resolution.

Three Approaches to Fuels from Fatty Compounds. G. Knothe, K.M. Doll, B.R. Moser, and R.E. Murray, USDA, ARS, NCAUR, USA.

Biodiesel, the alkyl esters, usually methyl esters, of vegetable oils, animal fats or other triacylglycerol-containing materials, are the most common approach to producing a fuel from the mentioned materials. This fuel is obtained by transesterifying the oil or fat with an alcohol, usually methanol, in presence of a catalyst such as alkoxide or hydroxide. In more recent years, a fuel probably best termed renewable diesel has been developed which simulates the composition of conventional diesel fuel. This fuel is obtained by hydrotreatment of the triacylglycerol-containing materials. Even more recently, a decarboxylation process of fatty acids was reported which provides a mixture of long-chain alkene isomers and other products with potential fuel properties. The three materials are compared regarding their production, composition and properties.

BIO 3/H&N 3.1: Biomodifications, Biomechanisms, and Biosafety

This session is sponsored in part by DuPont Nutrition& Health, Johnson & Johnson, and Oilseeds & Bioscience Consulting

Chairs: R.F. Wilson, Oilseeds & Bioscience Consulting, USA; and M. Picklo, USDA, ARS, USA

Dietary Seed Oil Effects on Kidney Oxylipins Reveal Surprising Effects of Fatty Acids. H. Aukema[1,2], [1]University of Manitoba, Canada, [2]Canadian Centre for Agri-Food Research in Health and Medicine, Canada.

Oxylipins are biologically active products of polyunsaturated fatty acids [e.g. eicosanoids derived from arachidonic acid (AA)]. Interestingly, the oxylipins formed from the two main polyunsaturated fatty acids in dietary seed oils, namely linoleic (LA) and a-linolenic acid (ALA), are almost completely unexplored. However, they are present in large amounts in animal tissues, they can have bioactivity similar to the other oxylipins, and diet significantly alters these oxylipins. In obesity-associated nephropathy, the oxylipins derived from ALA correlated with protection from the development of nephropathy. Furthermore, although the level of dietary LA did not change the levels of AA, the levels of AA (and LA) oxylipins were altered, demonstrating that fatty acid level does not necessarily predict potential biological effects. In another renal disease, the protective effect of dietary flax oil on disease progression was associated with amelioration of oxylipin abnormalities. Interestingly, despite the belief that ALA is poorly converted to docosahexaenoic acid (DHA), and the reduced DHA levels were not restored by flax feeding, dietary ALA was converted to DHA in sufficient amounts to restore the lowered DHA oxylipins in this disease. This suggests that the conversion of ALA to DHA is greater than is reflected by fatty acid levels.

Characterization of *Brassica napus* Type-1 Diacylglycerol Acyltransferase Variants Produced Through Directed Evolution. Y. Xu, G. Chen, and R.J. Weselake, University of Alberta, Canada.

Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of *sn*-1,2-diacylglycerol to produce triacylglycerol (TAG) and CoA. The level of DGAT activity during seed development appears to have a substantial effect on the flow of carbon into seed oil. Previously, directed evolution approach was used to generate an assortment of *Brassica napus* C.DGAT1.a (BnaC.DGAT1.a) variants which resulted in increased TAG content when recombinant forms of the enzymes were produced in *Saccharomyces cerevisiae* strain H1246. In the current study, microsomes were prepared from H1246 yeast lines, producing various recombinant Bna.C.DGAT1.a variants, at the mid-log phase of growth. The microsomal fractions were then analyzed for DGAT activity using radiolabeled substrate and BnaC.DGAT1.a polypeptide using Western blotting. The results of these assays indicated that the BnaC.DGAT1.a variants could be divided into three groups: 1) enzymes with increased activity; 2) enzymes which exhibited increased polypeptide accumulation; and 3) enzymes which were activated along with exhibiting increased polypeptide accumulation. The most promising variant, which resulted in a 2.3-fold increase in TAG content in yeast relative to the native form of the enzyme, belongs to category 3.

***In vivo* and *in vitro* Evidence for Biochemical Coupling of Reactions Catalyzed by Lysophosphatidylcholine Acyltransferase and Diacylglycerol Acyltransferase.** X. Pan[1], S. Stymne[2], J. Zou[3], X. Qiu[4], G. Chen[1], and R.J. Weselake[1], [1]University of Alberta, Canada, [2]Swedish University of Agricultural Sciences, Sweden, [3]National Research Council Canada, Canada, [4]University of Saskatchewan, Canada.

Flax (*Linum usitatissimum* L.) seed oil is highly enriched in a-linolenic acid (ALA). Phosphatidylcholine (PC) is the major site for ALA synthesis and thus efficient mechanisms are required to channel ALA from PC into triacylglycerol (TAG). The PC de-acylation reaction catalyzed by the reverse action of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) could potentially transfer ALA produced on PC directly into the acyl-CoA pool making this polyunsaturated fatty acid (PUFA) available for the diacylglycerol acyltransferase (DGAT)-catalyzed reaction for TAG production. Firstly, *in vivo* experiments showed that co-expressing flax *DGAT1* and *LPCAT* in a yeast quintuple mutant significantly increased 18-carbon PUFA in TAG with a concomitant decrease of 18-carbon PUFA in phospholipid. Secondly, *in vitro* experiments further showed that yeast microsomes containing both DGAT1 and LPCAT were able to

transfer [^{14}C]-labeled linoleoyl or linolenoyl moiety at a higher rate than oleoyl moiety from the *sn*-2 position of PC to TAG. Together, our data support the hypothesis of biochemical coupling of the LPCAT reverse reaction with the DGAT1 forward reaction for the incorporation of ALA into TAG. This process represents a possible mechanism for enriching TAG in PUFA during seed development in flax.

Preparation of High-purity DHA from Microalgae Oil in a Packed Bed Reactor via Two Step Lipase-catalyzed Esterification. E.J. Lee[1], D.S. No[1], M.W. Lee[1,2], and I.H. Kim[1], [1]Korea University, Republic of Korea, [2]Ilshinwells, Republic of Korea.

High-purity docosahexaenoic acid (DHA) was produced successfully in a packed bed reactor via two-step lipase-catalyzed esterification using the fatty acid from microalgae (from *Crypthecodinium cohnii*) and ethanol as substrates. Lipozyme RM IM from Rhizomucor miehei was employed as a biocatalyst. For the first reaction, several parameters including temperature, molar ratio of the substrates (fatty acid to ethanol), and water content in the substrate mixture (based on the total substrate weight) were investigated as a function of the residence time. A temperature of 40^{o}C, a molar ratio of 1:4, and a water content of 0.6% were selected as optimum conditions. Under these conditions, the maximum DHA concentration of over ca. 90% was achieved in the residual fatty acid fraction with an 89% yield. For the second reaction, the residual fatty acid separated from the first reaction product was used as a substrate. The optimum condition of the first reaction was used for the second reaction. After the second reaction, a DHA concentration of 100% was achieved in the residual fatty acid with a 94% yield.

Solvent-induced 7*R*-Dioxygenase Activity of Soybean 15-lipoxygenase-1 in the Formation of Omega-3 DPA-derived Resolvin Analogs. E.P. Dobson, C.J. Barrow, and J.L. Adcock, Deakin University, Australia.

The resolvin family contains important anti-inflammatory and pro-resolution compounds enzymatically derived *in vivo* from the omega-3 polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). More recently, docosapentaenoic acid (DPA) has emerged as another potentially important precursor in the biological production of resolvin compounds.

In this work we have used medium engineering to develop a simple method for the controlled synthesis of two di-hydroxylated diastereomers of DPAn-3 catalyzed by soybean 15-lipoxygenase-1 (15-sLOX-1) in the presence of short chain *n*-alcohols, including methanol, ethanol and propan-1-ol. The complete structures of the two major products – 7*S*,17*S*- and 7*R*,17*S*-dihydroxy-DPAn-3 have been elucidated using various chromatographic and spectroscopic analyses, including chiral HPLC, UV-visible, FTIR, and NMR spectroscopy. The alcohol-dependent *R*-dioxygenase activity of 15-sLOX-1 with mono-hydroperoxide intermediate substrates has also been demonstrated with other biologically relevant PUFAs, including DHA, EPA and arachidonic acid. The developed method has applications in the production of closely related isomers of naturally occurring resolvins and protectins, demonstrating the versatility of 15-sLOX-1 as a biocatalyst.

Effect of Dietary Lysophospholipids Containing n-3PUFAs on Serum and Liver Lipids Contents in Rats. R. Hosomi[1], K. Miyauchi[1], K. Fukunaga[1], Y. Inoue[2], T. Nagao[3], M. Yoshida[1], and K. Takahashi[4], [1]Kansai University, Japan, [2]Lipid Lab., Japan, [3]Osaka Municipal Technical Research Institute, Japan, [4]Hokkaido University, Japan.

The EPA and DHA have beneficial health properties, *i.e.,* decrease in serum triglyceride (TAG) and anti-platelet aggregation. Bioavailability of n-3PUFAs depends on their chemical form, such as TAG, ethyl-ester, fatty acid, and phospholipid. Superior bioavailability has been suggested for phospholipid containing n-3PUFAs. Currently, no information is available concerning the effect of lysophospholipids (Lyso-PLs) containing n-3PUFAs on lipid metabolism. In this study, we investigated the effects of Lyso-PLs containing n-3PUFAs on serum and liver lipids contents in rats. Lyso-PLs containing n-3PUFAs were prepared from squid meal, which is fisheries byproduct. Groups of male Wistar rats were fed AIN93G diet containing soybean oil (SO, 7%), TAG containing n-3PUFAs (2.0%) + SO (5.0%), and Lyso-PLs containing n-3PUFAs (2.0%) + SO (5.0%). The following indicators were assayed as indexes of lipid metabolism: TAG and cholesterol in serum and liver, fecal cholesterol, bile-acid excretion. Serum TAG and cholesterol contents decreased significantly in the group fed Lyso-PLs containing n-3PUFAs as compared with TAG containing n-3PUFAs, which had the same contents of n-3PUFAs as the Lyso-PLs diet. Therefore, Lyso-PLs containing n-3PUFAs has the

possible to be useful functional food materials.

Effect of Feeding DHA as Phospholipid, Triacylglycerol, or Both on DHA Concentration of Brain Regions, Liver, and Serum Lipids. A.P. Kitson[1], A. Berger[2], and R.P. Bazinet[1], [1]University of Toronto, Canada, [2]Arctic Nutrition, Norway.

DHA is important for neurological function, but the form of dietary DHA most bioavailable to the brain is not agreed upon. There is evidence that DHA in phospholipids (PL) is more bioavailable then triacylglycerol (TAG), however previous studies comparing PL- and TAG-DHA only examine acute brain uptake after one dose and have not examined differences in metabolism. This study compared DHA as TAG-rich fish oil (FO), PL-rich caviar PL concentrate (PLC), or a mixture of both (FO+PLC) on brain, liver, and serum DHA. After 11 weeks on a low omega-3 diet, male rats received 2% fatty acids as DHA as FO, PLC, or FO+PLC or an olive-oil (OO) supplemented diet for 4 weeks. DHA was higher in total lipids of cortex, cerebellum, hippocampus, brainstem, striatum, and rest of brain as well as liver in all DHA supplemented groups relative to OO controls with no significant differences between supplemented groups. Similarly, DHA-supplemented groups had higher DHA in serum PL, TAG, non-esterified fatty acids and cholesterylesters relative to OO with some evidence of higher serum DHA in FO+PLC-supplemented rats relative to other DHA groups. All forms of DHA appear equally effective at increasing tissue DHA, while combined PL+TAG-DHA may target serum DHA.

An Efficient Gene Targeting and Molecular Breeding in Oil-producing Fungus *Mortierella alpina* with Deletion of *lig4* Gene for Non-homologous End Joining. H. Kikukawa[1], E. Sakuradani[1,2], A. Ando[1], S. Shimizu[1], and J. Ogawa[1], [1]Kyoto University, Japan, [2]University of Tokushima, Japan.

The oil-producing zygomycete *Mortierella alpina* 1S-4 is known to accumulate beneficial polyunsaturated fatty acids (PUFAs), such as arachidonic acid (ARA) and eicosapentaenoic acid (EPA). To achieve high production of valuable PUFAs by metabolic engineering, an efficient gene deletion system, such as gene targeting, is necessary for PUFAs production by this fungus. Here, to develop an efficient gene targeting in *M. alpina* 1S-4, we identified the *lig4* gene encoding DNA ligase 4 involved in non-homologous end joining (NHEJ) on genomic double strand breaks repair, and then constructed *Δlig4* strain from *M. alpina* 1S-4. The replacement in the *Δlig4* strain was improved from 3% to 67% of gene targeting efficiency. In addition, the *Δlig4* strain showed no defect in vegetative growth and formation of spores. Furthermore, dihomo-γ-linolenic acid (DGLA)-producing strains were constructed with deletion of Δ5-desaturase (Δ5ds) gene encoding a key enzyme of bioconversion of DGLA to ARA using the *Δlig4* strain as a host strain. DGLA composition of the strains reached about 40%, and in contrast, ARA composition of the strains decreased. From these results, the *Δlig4* strain from *M. alpina* 1S-4 is available for general strain of metabolic engineering.

Effect of Harvest Time on Olive and Olive Oil Properties During Ripening for Gemlik and Adana Topagi Olives. T.M. Keceli, University of Cukurova, Turkey.

Harvest time plays a key role in the quality and oxidative stability of olive oil. Adana Topagi and Gemlik were harvested at three different times and the oils were obtained on a laboratory scale. The results showed that as the fruit matured, the oil became less stable due to decreasing total polyphenol content, increasing polyunsaturated (mainly linoleic acid), and decreasing chlorophyll content. While pomological properties of olive fruits and oil content increased total phenol, chlorophyll carotenoid content and antioxidant activity was decreased depending on harvest date for Adana Topagi and Gemlik olives ($p<0.05$). The best radical scavenging properties were obtained from Gemlik olives and Adana Topagi extracted olive oils. There was a strong interaction between total polyphenol content and DPPH radical scavenging activity for Gemlik and Adana Topagi olives. Chlorophyl and caretenoid content of Gemlik extracted olive oils were higher than Adana Topagi extracted olive oils during ripening ($p=0.05$). Gemlik olives were found to be effective antioxidants on DPPH inhibition and oxidative stability of refined olive oils ($p<0.05$). It was found that variety and harvest time has significant effect on both some physical, chemical properties and antioxidant activity of olives and their olive oils.

BIO 4/S&D 4: Biobased Surfactants/Detergents

Chairs: D.K.Y. Solaiman, USDA, ARS, ERRC, USA; D.G. Hayes, University of Tennessee, USA; G.A. Smith, Huntsman Performance Products, USA; and R.M. Maier, University of Arizona, USA

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

Modern day surfactants are based on natural, petrochemical or a combination of natural and petrochemical feedstocks. With the recent emphasis on sustainability, surfactants based on natural feedstocks are of considerable interest. Typically, natural surfactants are based coconut or palm oil. Both of these materials are also used as a food source. An alternative feedstock which is not used for food is algae. There are thousands of different algae species which can grow in fresh or salt water.

Work was performed to optimize the growth conditions for chlorella vulgaris algae in photobioreactors (PBR). Light frequencies and fertilizer concentrations were varied to achieve the optimum growth conditions. In order to maximize the lipid yield, the algae were stressed and the lipids extracted. The oil was used to make a variety of different nonionic surfactants. Surface properties and detergency measurements were performed.

Comparison of Performance for Sugar Esters Prepared by a Green Enzymatic Process and a Commercially Available Product. R. Ye[1], D.G. Hayes*[1], R.M. Burton[2], A. Liu[3], and Y. Wang[3], [1]University of Tennessee, USA, [2]MARC-IV Consulting, Inc., USA, [3]Tianjin University of Science and Technology, China.

Sugar esters, important biobased surfactants and emulsifiers for foods, cosmetics, pharmaceuticals, and other applications, are traditionally prepared in high-temperature processes, in the presence of organic solvents, and using a stoichiometric excess of reactant. We have successfully prepared sugar esters using green manufacturing: immobilized enzymes, the absence of solvents, and stoichiometric substrate feeds. Therefore, the green manufacturing approach will potentially yield a product meeting user specifications without the need of downstream purification, and the absence of wastes. In this study, the chemical composition, surface and antimicrobial activity of the enzymatically prepared sugar esters were measured, and compared to a commercially available sugar ester product. Typically, the authors' bioreactor system yields a technical grade product, ~85-90% pure, at the 10-30 gram scale. To achieve a higher purity. The technical grade product underwent further esterification, in a very low water activity environment, achieved by operating the reaction in well-sealed dessicators in the presence of dessicant. The study will compare chemical composition and molecular structure, surface and interfacial tension, emulsification, and antimicrobial activity.

A New and Cost-effective Biosynthetic Process for Hydroxylated PUFA's by the Yeast *Starmerella bombicola*: Opportunities for Bio-medical Research. I.N.A. Van Bogaert[1], G. Zhang[2], B. Hammock[2], and W. Soetaert[1], [1]Ghent University, Belgium, [2]Bruce Hammock Lab, USA.

Poly-unsaturated fatty acids (PUFAs) are essential in human nutrition as they take part in various key signaling pathways. In general, not the PUFAs themselves trigger these effects, but their oxidized or hydroxylated derivatives. Yet, such compounds are rare and if commercially available extremely expensive, in this way hampering *in vivo* experiments. We developed a whole-cell protocol for the generation of ω and ω-1 hydroxylated PUFAs based on the yeast *Starmerella bombicola*.

This yeast is known for its ability to produce sophorolipids, a biosurfactant of commercial interest applied in environmentally friendly cleaning formulations. Biosynthesis involves the action of the cytochrome P450 monooxygenase CYP52M1 hydroxylating C16-18 fatty acids at the terminal or subterminal position. Interestingly, unsaturated fatty acids with 20 and even 22 carbon atoms get hydroxylated as well.

Consequently, the sophorolipid biosynthetic route was used to hydroxylate various PUFAs. The hydroxylated molecules are incorporated into the sophorolipids and secreted in the culture medium, allowing efficient recovery. They can be released from the sophorolipid molecules by acidic hydrolysis. Yield was substantially increased utilizing a modified yeast strain (0.01 vs 20 % after purification).

Production of Biosurfactants Using *Bacillus subtilis* on Pretreated Biomass Hydrolysates in 5-L Bioreactor. R. Sharma, W.J. Colonna, and B.P. Lamsal, Iowa State University, USA.

Surfactin is a *Bacillus subtilis* biosurfactant which has excellent surface active properties but has limited aqueous solubility.Recombinant strain of surfactin producing *B. Subtilis*(E4088), produces a water soluble variant of surfactin called FA-glu due to a less hydrophobic amino acid structure than surfactin. In a study for 50-mL shake flask fermentation for both strains, soy hulls, alfalfa, and switchgrass were chosen as the best carbon sources compared to glucose for highest growth and product concentration for FA-glu and surfactin producing *Bacillus subtilis* strains. These 3 biomasses were pretreated with a combination of liquid ammonia and ultrasonication among six fibrous biomasses to generate hydrolyzates, utilized as carbon source in growth media for both *Bacillus* strains in 50-mL shake flask experiments. It was observed that glucose content and relative availability of hexose and pentose sugars in the hydrolysates played an important role in determining highest growth and product titer. We have designed 5-L fermentations of growth media based on these selected biomass hydrolysates as carbon source to study bacterial growth, economic efficiency and product quality of the surfactin and FA-Glu which would provide a comparative analysis of scale up, process kinetics and reactor performance.

Challenges to Realizing the Commercial Potential for Biosurfactants. R.M. Maier, University of Arizona, USA.

Since their initial discovery and introduction to the marketplace in the 1960's, biosurfactants have experienced a steady rise in interest and potential applications as "green" alternatives to traditional petroleum-based specialty surfactants. This is due to a combination of their environmentally friendly characteristics, including low toxicity and ready biodegradability, as well as their excellent surfactant attributes. This presentation will discuss some of the challenges associated with realizing the market potential for biosurfactants. There are still relatively few biosurfactant structures that have been well characterized in terms of their structure, surfactant properties, biosynthesis, regulation, or encoded genetic information. This process is tedious and difficult and further complicated by the fact that it is a combined microbiology and chemistry problem that few laboratories are well-equipped to carry out. An even larger challenge is how to cost-effectively purify these materials for use as specialty chemicals. Biosurfactants are often produced as congener mixes which can lead to batch to batch variability in production that may not be acceptable in a specialty chemical. Finally, discovery of new biosurfactants is limited because currently available molecular screening techniques are of little use and manual culture-based searches are expensive in terms of time and labor.

Tailoring Rhamnolipid Biosurfactant Properties Through Production by Chemical Synthesis. J.E. Pemberton, R. Palos-Pacheco, C.S. Coss, and R. Polt, University of Arizona, USA.

Rhamnolipids are an important class of biosurfactants being explored as greener alternatives to petroleum-based surfactants. These surfactants are generally comprised of a ß-hydroxyalkanoyl alkanoate tail glycosylated by one or two rhamnose units, with the most abundant containing a ß-hydroxydecanoyl deacanoate tail (Rha-C10-C10 or Rha-Rha-C10-C10). To date, attainable rhamnolipid properties have been limited to those of the biosynthesized materials. We use a high-efficiency, cost-effective approach to chemical synthesis that allows production of novel rhamnolipids whose properties can be tailored through control of molecular structure. Here, characterization of a series of monorhamnolipids whose combination of tail lengths is selected to vary the molecular shape from cylindrical to conical is described; Rha-C14-C14, Rha-C14-C12, Rha-C14-C10, Rha-C14-C8, and Rha-C14-C6 have been prepared and studied. In contrast to the expected monotonic change in surfactant properties (e.g. CMC, minimum surface tension, micelle aggregation number) with shape, minima (or maxima) in these metrics are observed across this series of molecules indicating a complex interplay of multiple variables that dictate surfactant properties. These results demonstrate the power of molecular design and chemical synthesis for harnessing the potential of rhamnolipid surfactants.

Scaling Up Rhamnolipid Production: Comparison of Flask, Bench, Pilot, and Demo Scale Fermentations. D. Derr, N. Lohitharn, R. Mirani, and P. Tedrick, Logos Technologies, USA.

Rhamnolipids (RL's) are a promising class of biosurfactants that have achieved only limited commercial success to date. At pH's above their pKa, RL's are anionic surfactants made up of rhamnose

sugar units and medium chain length 3-hydroxy fatty acids. A terminal carboxylic acid provides the anionic functionality. RL's can be produced in a fermentation process using bacteria that excrete the product. This allows straightforward product isolation after fermentation is complete. Two reasons commonly given for RL's lack of commercial success are (1) high cost of production and (2) difficulty in scaling up production processes from the flask scale to commercially relevant scales. In this talk we will briefly discuss a simple, inexpensive method to produce rhamnolipids and then show results from that process at several scales. First we will discuss transitioning from flask scale into small, 1 L working volume, fermenters. This is the barrier that is often hardest to cross in fermentation processes, and we do show a decrease in production metrics. However, as fermentation volume increases, to 2 L, and then 15 L working volume, metrics improve again, and are commercially viable. Expansion to 300 L scale will also be discussed.

Use of Bioenhancers to Improve Growth and Product Quality of Biosurfactants. R. Sharma and B.P. Lamsal, Iowa State University, USA.

Biosurfactants produced through bacterial fermentation have been grown on a variety of sugars and carbohydrate sources. The titers for products formed, however are limited due to inefficient utilization of sugars and nutrients in the media. In this study we aim to study the effect of two bio-enhancers, a) Baclyte© and b) norepinephrine for heightened growth for two well-known biosurfactants: surfactin and rhamnolipids. Baclyte©, a proprietary growth enhancer for gram positive *Bacillus subtilis*, has shown to significantly increase growth with glucose as the main source of carbon in the media. Norepinephrine has also shown to increase the growth of rhamnolipid producing *Pseudomonas aeruginosa* through increased quorum sensing among populations of the bacterium in a glucose rich environment. We have designed bioscreen scale optimization studies for different combinations of sugar and these two bioenhancers for each bacterial strain to test for highest bacterial growth and product formation. The study includes combinations of glucose, sugars mimicking cellulose compositions in biomasses, biomass hydrolyzates at different concentrations (0-5% w/v) in combination with varying concentrations of the two enhancers (0-3% w/v). Initial results for baclyte in combination with glucose for *Bacillus subtilis* have shown accelerated growth and sustained peak absorbance values.

Sophorolipids—The Next Leading Class of Surfactants. D. Kuppert[1], A. Nagy[2], and G. Tian*[2], [1]Evonik Industries AG, Germany, [2]Evonik Corp., USA.

The limited availability of petrochemical feedstock for surfactants demands a change to sustainable raw materials. Mother Nature provides a plethora of renewable and vegetable-based raw materials like carbohydrates or vegetable oils, which are excellent starting materials for biosurfactants. Sophorolipids, a group of biosurfactants with an unique and sophisticated structural composition, are capable of replacing petrochemically based surfactants. There is a need to produce high quality sophorolipids at reasonable costs.

We have been working on an effective fermentation process including down-stream processing to make sophorolipids available on an industrial scale. In this process, sophorolipids are made by a biological process using yeast with a high carbon uptake. They have an excellent toxicological and eco toxicological profile and are completely biodegradable. Moreover, sophorolipids are very gentle to human skin. All three aspects are excellent prerequisites for their usage in typical household care cleaning applications, such as hand dish wash liquids, hard surface cleaners or degreasers. It has been demonstrated that the switch from chemically made surfactants to sophorolipids in such formulation is possible without the loss of performance. Even superior application properties can be achieved with sophorolipids.

Biosurfactants as a Tool for Metal Removal from Waste Effluents. D.E. Hogan, J.E. Pemberton, and R.M. Maier, University of Arizona, USA.

Mining and industrial effluents contain valuable elements at very low concentrations. We are investigating the potential for use of the biosurfactant rhamnolipid produced by *P. aeruginosa* for the recovery of these metals. To determine the conditional stability constants for natural and synthetic monorhamnolipid with a variety of elements, a resin-based ion exchange method paired with inductively coupled plasma optical emission spectrometry was used. Of the metals tested, rare earth elements have the greatest stability constants with natural rhamnolipid. The conditional stability constant sequence for 25 metals tested (from strongest, log K = 9.77, to weakest, 0.96) is Eu^{3+} > Nd^{3+} > Tb^{3+} > La^{3+} > Al^{3+} > Cu^{2+} > Pb^{2+} > Y^{3+} > Pr^{3+} > Dy^{3+} > Lu^{3+} > Cd^{2+} > In^{3+} > Zn^{2+} > Fe^{3+} >

$Hg^{2+} > Ca^{2+} > Sr^{2+} > Co^{2+} > Ni^{2+} > Cs^{+} > Ba^{2+} > Mn^{2+} > Mg^{2+} > K^{+}$. Data showed rhamnolipid selectively removes metals with high log K values over those with low values. Four synthetic rhamnolipid diastereomers were studied for effect of molecular orientation on metal interactions. Different arrangements at rhamnolipid's two stereocenters affected the log K values for Pb and Cd relative to natural rhamnolipid.

BIO 5: General Biotechnology

Chairs: R.D. Ashby, USDA, ARS, ERRC, USA; and B.P. Lamsal, Iowa State University, USA

Milk Fat Triacylglycerol Profile Differentiation Between DGAT1 AA and KK Genotype: Effect of Fat Supplementation. D.A. Tzompa-Sosa, H. Bovenhuis, A.M. van Vuuren, and H.J.F. van Valenberg, Wageningen University, The Netherlands.

Milk fat triacylglycerols (TAG) determine the physical and functional properties of butter and products rich in milk fat. Milk fat TAG profile is affected by dietary lipids and could also be genetically determined but so far, no genetic effect has been studied. The aim of this study was to determine the effect on milk fat TAG profile of dietary lipids with two contrasting FA profile and the effect of DGAT1 K232A polymorphism, an enzyme related to TAG synthesis. Eight multiparous Holstein-Friesian dairy cows were used, two carrying DGAT1 AA and two carrying KK genotype. Linseed oil and palm fat were supplemented intra-ruminally to each cow in two different periods. Milk from one day was pooled and the fat was separated. Milk fat TAG profile was analysed by positive-ion MALDI-TOF using DHB as matrix. Cluster analysis and heat maps were used to analyse the results. Supplementation of fats and oils increased high molecular weight TAG, however linseed supplementation resulted in a higher amount of unsaturated TAG. This difference was enough to differentiate between oil supplementation. Moreover, DGAT1 K232A polymorphism affects the milk fat TAG profile. The effect is more evident in KK genotype than in AA genotype. The main change is happening in TAG with 39 carbons.

Natural Products Produced from Bioethanol Stillage. K. Ratanapariyanuch[1], Y.Y. Shim[1,2], M. Haakensen[3], and M.J.T. Reaney[1,2,4], [1]University of Saskatchewan, Canada, [2]Prairie Tide Chemicals Inc., Canada, [3]Contango Strategies Ltd., Canada, [4]Jinan University, China.

Thin stillage (TS), liquid waste from ethanol industry, is a mixture of macromolecules (protein and polysaccharides), organic compounds (ethanol, isopropanol, lactic acid, 1,3-propanediol, acetic acid, glycerophosphocholine, betaine, glycerol, and phenethyl alcohol) and inorganic molecules (sodium, potassium, and sulfate). It was observed that micro-organisms especially *Lactobacillus panis* strain PM1, isolated from fermenting TS, had ability to convert glycerol and lactic acid to 1,3-propanediol and acetic acid, respectively. In addition, it was discovered conversion could be improved by adjusting pH to 5 under oxygen depleted condition along with supplementing with glucose (0.1 M), glycerol (1 M), vitamins (B2, B3, and B12), and freeze-dried stillage. While fermentation at 25°C required 96 h for effective conversion of glycerol to 1,3-propanediol (>60% yield) raising the fermentation temperature to 37°C enabled this conversion to occur in just 48 h. Through bacterial fermentation the ethanol industry is a promise source of commodity platform chemicals.

Enzymatic Synthesis of High Oleic Oil Based Structured Lipid Containing Palmitic and Capric Acid Suitable for a Human Milk Fat Substitute. C. Álvarez and C.C. Akoh, University of Georgia, USA.

For those health conditions where infants cannot be breastfed, commercial formulas are the most convenient alternative. However, the differences in stereospecific structure of triacylglycerols (TAGs) in vegetable oils used in infant formula relative to those in human milk fat lead to lower energy use and less calcium absorption by formula-fed infants. The aim of this study was to produce a structure lipid (SL) with a substantial amount of palmitic acid esterified at the *sn*-2 position as present in human milk fat. In addition, capric acid was incorporated in the SL as a readily available source of energy for the growing infant. The SL was produced by a two-step enzymatic interesterification of high oleic sunflower oil (HOSO), high melting point palm stearin (HMPS, m.p=53°C) and tricaprin (TC). The enzyme used was the *sn*-1,3 specific Lypozyme TLIM lipase. The final product synthesized contained ~40 mol% of palmitic acid at the *sn*-2 position, ~20 mol % of total capric acid, and ~50 mol% of total oleic acid. The product yield for the reaction was 90%. The SL produced may be used for formulating a commercial infant formula closer to the composition of mother´s milk.

***In situ* Self-catalyzed Transesterification for Production of Biodiesel from Rice Bran.** N. Choi, D.S. No, and I.H. Kim, Korea University, Republic of Korea.

As an innovative strategy, self-catalyzed transesterification in rice bran was carried out successfully for the production of biodiesel. With this

method, biodiesel was synthesized from the oil in rice bran by simply adding methanol to rice bran, with the aid of rice bran lipase already existing in the rice bran. For the optimization of the reaction, the effect of reaction temperature, molar ratio (fatty acid to methanol) and the water content of the rice bran were investigated. To determine the effects, the yield of biodiesel as well as the free fatty acid content was monitored as a function of reaction time. The optimum conditions were found to be the temperature of 40°C, the molar ratio of 1:2, and the water content of 12wt%. After the 12 days of reaction, the biodiesel yield of 83.4wt% was obtained. In order to increase the yield of biodiesel further, re-transesterification was conducted with the reaction product obtained from the first transesterification. Finally, 100% conversion of the saponifiable lipids in rice bran was confirmed and the maximum yield of 95.2wt% was obtained after 6 days of re-transesterification.

Application of IPA-Water-Oil, IPA-Methanol-Oil, and IPA-Water-Salt Ternary Phase Diagrams in Biodiesel Production from Mustard Seed. S. Sinichi and L. Diosady, University of Toronto, Canada.

Isopropyl alcohol (IPA) was used in developing a rapid method for oil extraction from yellow mustard to recover oil suitable for conversion to biodiesel. The recovery of IPA after oil extraction and biodiesel production was investigated to improve the efficiency and reduce the solvent cost. Oil extraction, mix alcohol transesterification and IPA recovery processes were modelled after developing the ternary phase diagram of IPA-water-oil and IPA-methanol-oil as well as IPA-water-salt to characterize the process conditions. Multi-stage extraction improved oil recovery with up to 93.7% oil yield using 4-stage extraction at 2:1 IPA:flour (volume:weight) ratio at room temperature. IPA extracted mustard oil was converted to high quality esters with a 99% yield. As IPA forms a stable azeotrope with water improvements in IPA recovery were attempted by salting out IPA from the azeotrope with K_2CO_3. By using 20% K_2CO_3 (w/w of the mixture), 95% of the IPA was recovered at ~99% purity. The overall results suggest that IPA extraction followed by IPA recovery using salt enhanced phase separation is technically viable for near complete recovery of the oil and protein as well as recycling of IPA.

Novel Linoleic Acid Δ 12 Hydratase from *Lactobacillus acidophilus* Useful for 13-hydroxy Fatty Acid Production. S. Kishino, A. Hirata, and J. Ogawa, Kyoto University, Japan.

Hydroxy fatty acids are widely used in chemical, medical and cosmetic industries. Recent studies have indicated that several hydroxy fatty acids have anti-inflammatory effects and improve lipid metabolism. Through the screening for ability to convert linoleic acid (LA) into hydroxy fatty acids in lactic acid bacteria, we found that *L. plantarum* converted LA into 10-hydroxy-12(*Z*)-18:1, and succeeded in the identification of the hydratase, named CLA-HY, responsible for this conversion. *L. acidophilus* NTV001 converted LA into mainly 13-hydroxy-9(*Z*)-18:1. We investigated the genome information of *L. acidophilus* strains, and found that there were two genes with homologous protein sequences with that of CLA-HY in *L. acidophilus*. We amplified the candidate genes with *L. acidophilus* NTV001 genomic DNA as a template and transformed into *E. coli*. Product analysis of the reaction mixtures with *E. coli* cells expressing each gene and LA revealed that one gene product generated 10-hydroxy-12(*Z*)-18:1, a Δ9-hydration product, and the other gene product, named FA-HY, generated 13-hydroxy-9(*Z*)-18:1, a Δ12-hydration product. Furthermore, we found that FA-HY was able to convert LA, a-linolenic acid, Δ-linolenic acid, and stearidonic acid into corresponding 13-hydroxy fatty acids through Δ12-hydration.

The Biochemistry of Two Microalgae with Potential as n-3 PUFA Producers. J.L. Harwood[1], I.A. Guschina[1], and K.J. Flynn[2], [1]Cardiff University, UK, [2]Swansea University, UK.

There is an increasing need for n-3 polyunsaturated fatty acids, the bulk of which have traditionally been supplied by fish oils. However, the primary producers are photosynthetic microalgae. Two such marine algae which are currently used in aquaculture and which have been suggested to have further commercial potential as n-3 PUFA producers are *Nannochloropsis oculata* and *Phaeodactylum tricornutum*.

We have been studying the lipid biochemistry of these two algae so as to identify features of importance in increasing the amount and n-3 PUFA content of the endogenous lipids. In particular, we have used metabolic radiolabelling to follow the primary reactions of *de novo* synthesis, desaturation and elongation. Analytical methods include lipid class separation, radio-glc of acyl components and

lipidomics using MS/MS. The main lipids of both algae are the glycosylglycerides and phosphatidylcholine and phosphatidylglycerol. The role of these complex lipids as substrates for desaturation in the production of eicosapentaenoic acid (EPA) in particular will be discussed. Furthermore, differences in metabolism and potential control points in lipid accumulation will be highlighted.

Preparation of Stearidonic Acid-rich Triacylglycerol via Two-step Lipase-catalyzed Esterification. N.H. Kim, J.Y. Baik, and I.H. Kim, Korea University, Republic of Korea.

The aim of this study was to synthesize stearidonic acid (SDA, 18:4n-3)-rich triacylglycerol (TAG) via a two-step lipase-catalyzed esterification under vacuum. SDA-rich fatty acid which was used as a substrate for synthesis of SDA-rich TAG was prepared from echium seed oil via *Candida rugosa* lipase-catalyzed selective esterification. Two different immobilized lipases, such as Novozym 435 from *Candida antarctica* and Lipozyme TL IM from *Thermomyces lanunginosus*, were employed for the synthesis of SDA-rich TAG. For the first step, Novozym 435-catalyzed esterification of SDA-rich fatty acid and glycerol was carried out for the synthesis of TAG during the short reaction time of 2h. For the second step, Lipozyme TL IM-catalyzed esterification with reaction mixture from the first step was performed during the long reaction time of 30 h. Optimum reaction condition for the first step using Novozym 435 was a temperature of 60°C, an enzyme loading of 10%, and a vacuum of 0.7kPa, respectively, whereas that for the second step using Lipozyme TL IM was a temperature of 65°C, an enzyme loading of 20%, and a vacuum of 0.7kPa, respectively. Under these optimum conditions, a maximum TAG yield of ca. 93% was obtained after 32h via the two-step lipase-catalyzed esterification.

BIO 5.1/H&N 5.1/SCC: Lipid Oils and Skin Health

This session is sponsored in part by Johnson & Johnson

Chairs: T.A. McKeon, USDA, ARS, WRRC, USA; K. Mahmood, Johnson & Johnson Consumer, USA; and K. Dobos, Society of Cosmetic Chemists/Sun Chemical Corp., USA

The Role of Lipids in Skin Physiology. A. Pappas, Johnson & Johnson, USA.

The skin is the largest organ of the human body, which serves functions in thermoregulation, protection, metabolism and in sensory. Various lipids are fundamental for normal skin functions and are unusual as they are not found anywhere else in the human body. Sapienic acid, triglycerides, waxes and squalene are secreted by glands imbedded in the human skin and are deposited on the surface of the skin. These lipids contains an unusual mixture of fatty acids. Recently, the importance of all these lipids was further validated in animal models where the biosynthesis of fatty acid esters, triglycerides, long chain fatty acids and oleic acid has been impaired. All these animal studies together with recent reports on the effect of the dietary fat on skin, further demonstrate the importance and essential role of fatty acid metabolism in normal skin physiology. Vegetable oils, fruit extracts and their components: fatty acids, tocopherols, polyphenols are constantly used in a wide variety of topical consumer products and their effects on skin care and on dermal health had been seriously underestimated.

New Insights into the Role of Polyunsaturated Fatty Acids in Skin Physiology and Pathology. H. Gallagher, I.A. Guschina, D. Ramji, and J.L. Harwood*, Cardiff University, UK.

Ever since skin defects revealed the need for essential fatty acids in the diet, the important role of PUFA in the biochemistry of epidermal layers has been acknowledged. Interest has also included disease such as atopic eczema and psoriasis where certain dietary PUFA can alleviate symptoms in both animal models and human subjects.

One of the PUFAs tested is the n-6 acid, dihomo-gamma-linolenic acid (DGLA). Like n-3 PUFAs such as EPA or DHA, DGLA can be metabolised to anti-inflammatory compounds – in this case, prostaglandin E_1 or 15-hydroxyeicosatrienoic acid. Moreover, DGLA has been suggested as a compound of key importance in diseases such as atopic dermatitis. We have studied the metabolism and effects of DGLA using a number of tissue culture systems. DGLA is taken up rapidly, esterified into a variety of membrane lipids and has rapid effects on a number of inflammatory mediators. It is oxidised by both cyclooxygenase and lipoxygenase enzymes whose products are readily detected by MS. At the same time, its provision reduces the relative amount of its major metabolic product, arachidonate. This may be important since arachidonate is an n-6 PUFA which produces mainly inflammatory eicosanoids.

Biosynthesis and Skin Health Applications of Antimicrobial Glycolipids. D.K.Y. Solaiman and R.D. Ashby, USDA, ARS, ERRC, USA.

Microbial-produced glycolipids (MGLs) such as sophorolipids (SLs), rhamnolipids (RLs), and mannosylerythritol lipids (MELs) are amphiphilic molecules, and thus have been widely explored for use as surfactants/detergents, emulsifiers, and lubricants. A major hindrance to their widespread commercial adoption is the higher prices in comparison to their non-renewable petroleum-based counterparts. To overcome this, research abounds in developing production systems that will lead to lower production costs. On top of that, many studies have been and continue to be conducted to find and explore any value-added properties possessed by these molecules which could help justify the higher costs of MGLs. This paper will first survey the current advances in production trends and skin health applications (e.g., antimicrobial, woundhealing promotion, fibroblast rejuvenation, emollient, etc.) of MGLs. We will then present our lab's research on using surplus agricultural byproducts as inexpensive feedstocks in the fermentative production of MGLs, and provide evidence for SLs as antibacterial agents particularly by demonstrating anti-acne activity against the causative bacterium (*Propionibacterium acnes*) when immobilized on biopolymer films.

Meadowfoam (*Limnanthes alba*) Natural Products Inhibit Matrix Metalloproteinases in Human Keratinocytes: Relevance to Skin Health. C.L. Miranda, R.L. Reed, A.K. Indra, and J.F. Stevens*, Oregon State University, USA.

Meadowfoam (*Limnanthes alba*) is an oilseed crop which is grown in the Willamette Valley of Oregon. Meadowfoam seed oil has commercial value

as an ingredient of cosmetic products. A waste product of the oilseed extraction, the seed meal is a rich source of the glucosinolate, glucolimnanthin. We developed a patented procedure for enzymatic conversion of glucolimnanthin into the bioactive products, 3-methoxybenzyl isothiocyanate (MBITC) and 3-methoxyphenyl acetonitrile (MPACN), by fermentation of meadowfoam seed meal. We investigated the anti-inflammatory potential of these products in human skin cells with the aim to explore their use as meadowfoam oil additives to improve skin health. Skin keratinocytes overexpress pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs) in response to stimulation by UV light, chemical irritants and infection. We found that an extract of fermented meadowfoam seed meal and especially MBITC (at 1 µM), but not MPACN, inhibit MMP-9 protein expression and activity in stimulated HACAT keratinocytes without signs of cytotoxicity. These findings demonstrate the beneficial effects of a fermented meadowfoam seed meal extract and MBITC as additives to cosmetic oils for the prevention of skin inflammation through inhibition of MMPs.

Cosmetic Applications of Castor Oil and Its Derivatives. T.A. McKeon and X. He, USDA, ARS, WRRC, USA.

Castor oil is unique - it contains 90% of the hydroxy fatty acid ricinoleic acid. The hydroxy group imparts unique physical, chemical and physiological properties on castor oil. While best known for its laxative effect, castor oil derived products include lithium grease, engineering nylons and cosmetics. Castor oil is a common constituent of lipsticks and cosmetic creams. Derivatives of castor oil also are effective as anti-microbials, and may be used to preserve cosmetics from microbial deterioration. The castor oil seed contains potent allergens and the toxin ricin in the protein fraction. We evaluated the possibility that ricin might be present in castor oil. We obtained samples of castor oil subjected to different levels of processing and from commercial sources. After extraction of the oil with phosphate-buffered saline, we tested the aqueous extracts for ricin content. We detected 35ng/ml of ricin (lethal dose ˜2-5µg/kg) in samples derived from the cold-pressed castor oil supplied and from a commercially purchased sample. However, no ricin was detected in USP grade castor oil, neutralized or hydrolyzed castor oil. The presence of ricin in the cold-pressed oil could indicate that allergen is also present, indicating that use of cold-pressed castor oil for ingestion or topical application be avoided.

Production of Structured Phospholipids using Phospholipase and Lipase. S.H. Yoon, Woosuk University, Republic of Korea.

The transesterification abilities of microbial phospholipase A2 and lipase in organic solvents were studied. Phosphatidylcholine and caprylic acid were transesterified by incubation in organic solvents using phospholipase A2 and lipase. Caprylic acid was incorporated into the *sn*-1 and -2 positions at a rate of 87.7% using phospholipase A2 in hexane, and 36.7% using lipase in diethyl ether. Higher acyl migration into *sn*-2 was observed in diethyl ether than in hexane during transesterification using lipase, however, there was no substantial difference in the caprylic acid content at the *sn*-2 position. Acyl migration during transesterification in methanol was lower than in other organic solvents.

Preparation of Highly Purified Pinolenic Acid from Pine Nut Oil via Three-step Lipase-catalyzed Esterification. H.J. Kim[1], T.T. Zhao[1], D.S. No[1], C.T. Kim[2], and I.H. Kim[1], [1]Korea University, Republic of Korea, [2]Korea Food Research Institute, Republic of Korea.

Pinolenic acid (PLA) from pine nut oil was successfully enriched by three-step lipase-catalyzed esterification. The fatty acids present in pine nut oil were selectively esterified with ethanol using Lipozyme RM IM from *Rhizomucor meihei* as a biocatalyst and PLA was enriched in the fatty acid fraction. The optimum conditions of molar ratio of the substrate (fatty acid to ethanol) and temperature were 1:7 and 25°C, respectively. There was no significant effect in the enrichment of PLA when water was added in reaction mixture. The same protocol and optimum conditions were employed for second and third step lipase-catalyzed esterifications. For first step lipase-catalyzed esterification, PLA was enriched up to 41.6mol% from an initial value of 13.5mol% in the pine nut oil. Using PLA enriched fatty acid obtained from first step as a substrate, PLA was enriched up to 68mol% via second step lipase-catalyzed esterification. Consequently, a maximum PLA content of ca. 78mol% was obtained via third step lipase-catalyzed esterification.

BIO-P: Biotechnology Poster Session

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

1. **Enzymatic Preparation of L-a-Glycerylphosphorylcholine via Phospholipase A_1-Catalyzed Hydrolysis of Soy Phosphatidylcholine in Organic-aqueous Media.** H.J. Bang[1], I.H. Kim[2], and B.H. Kim*[1], [1]Chung-Ang University, Republic of Korea, [2]Korea University, Republic of Korea.

The aims of this study were to model Phospholipase A_1 (PLA_1)-catalyzed hydrolysis of soy phosphatidylcholine (PC) in hexane-water media for the production of L-a-Glycerylphosphorylcholine (L-a-GPC) and to optimize the reaction conditions using response surface methodology. The reactions were performed in a stirred batch reactor using Lecitase Ultra, a commercial fungal PLA_1. The effects of temperature (Te), reaction time (RT), water content (WC), and enzyme loading (En) on L-a-GPC content in the reaction products were elucidated using the models established. Optimal conditions to completely hydrolyze PC to L-a-GPC were: Te, 50°C; RT, 30h; WC, 69wt% of PC; and En, 13wt% of PC. The products obtained under these conditions contained 21.0wt% L-a-GPC, 71.1wt% FFA, and 7.9wt% phosphocholine, but were free of PC and LPC. The FFA and phosphocholine were completely removed using a diethyl ether extraction method and a silica column chromatography with a mixture of methanol and water as an eluent, respectively.

2. **Microbial Production of Biofuel from Saccharified Plant Biomass by Pentose Assimilating Thraustochytrids.** A. Matsuda[1], H. Nagatomo[1], A. Fujimoto[1], Y. Taoka[1], T. Matsuda[2], Y. Izumi[2], and M. Hayashi[1], [1]University of Miyazaki, Japan, [2]Biomaterial in Tokyo, Japan.

For a biofuel production from plant biomass, novel pentose assimilating thraustochytrids were isolated. In the present study, the influence of culture conditions on the lipid profile and the growth of the strains and the microbial production of biofuel from saccharified corn fibre were investigated.

As the pentose assimilating thraustochytrids, 60 strains were isolated in Japan by a pine pollen bating method using the medium containing pentose as a sole carbon source. The compositions and contents of fatty acid in the isolates were determined by GC analysis of their total lipids. The phylogenic analyses based on the 18S rRNA gene sequences were also carried out for an identification of the isolates. As a result of screening, 4 thraustochytrids that can grow on the medium containing pentose as a sole carbon source were isolated from the collected sea water and sea sand. These strains contained a large amount of fatty acids, such as n-3 docosahexaenoic and n-6 docosapentaenoic acids in the cells. All of the strains were identified tentatively as Aurantiochytrium sp. by the phylogenic analysis based on the 18S rRNA gene sequences. In the present study, the microbial production of biofuel from saccharified corn fibre using 2L-jar fermenters was also investigated.

3. **Microbial Production of Polyunsaturated Fatty Acids from High Concentration of Glycerol by *Aurantiochytrium* sp. mh2112.** A. Fujimoto, A. Matsuda, H. Nagatomo, Y. Taoka, and M. Hayashi, University of Miyazaki, Japan.

For polyunsaturated fatty acids (PUFAs) production from the waste of biodiesel production, novel thraustochytrids assimilating high concentration of glycerol were isolated. In the present study, the influence of culture conditions on the lipid profile of these strains were investigated.

As isolation sources of thraustochytrids, dead leaves, coastal sea water, sea sand, and piece of coral were collected in Japan. The novel thraustochytrids were isolated by a pine pollen bating method using the medium containing glycerol as a sole carbon source. The medium for the isolation contained 10% of glycerol as a carbon source in half strength of artificial sea water. Their fatty acid compositions and contents were determined by GC analysis of their total lipids. The phylogenic analyses based on the 18S rRNA gene sequences were also carried out for an identification of the isolates.

As a result of screening, 5 strains of thraustochytrids that can grow on the medium containing 10% of glycerol were isolated. These strains contained a large amount of PUFAs, such as n-3 docosahexaenoic and n-6 docosapentaenoic acids in the cells. In the present study, influence of culture conditions on the contents and compositions of the fatty acids in the cells were also investigated.

4. **Distribution of Polyunsaturated Fatty Acids in the Phospholipids of Thraustochytrids.** H. Nagatomo, A. Fujimoto, A. Matsuda, Y. Taoka, and M. Hayashi, University of Miyazaki, Japan.

Thraustochytrids accumulate marked amount of lipids, such as polyunsaturated fatty acids (PUFAs), in the cells. Although the microbial production of PUFAs as the form of triglycerides (TG) by thraustochytrids has been investigated, there are few information of PUFAs in phospholipids (PL) of thraustochytrids. In the present study, the PL class compositions and the distribution of PUFAs in each PL class were determined.

Several strains of Aurantiochytrium, Thraustochytrium, Schizochytrium, Ulkenia, and Parietichytrium were cultivated in the medium contained 3% of glucose, 1% of yeast extract, vitamins mixture, and metal ions mixture in half strength of artificial sea water (pH=7.0). Total lipids were extracted by Bligh and Dyer method. The phospholipids were separated from total lipids by column chromatography and fractionated by thin layer chromatography. The compositions and contents of fatty acid in the isolates were determined by GLC analysis after methanolysis of their total lipids.

In Aurantiochytrium, Thraustochytrium, Schizochytrium, Ulkenia, and Parietichytrium cells, major PL class were phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine. Higher docosahexaenoic acid were contained in PL than TG.

5. **Investigating the Anaerobic Peroxidase Activity of Soybean 15-lipoxygenase with LC-PUFAs.** E.P. Dobson, C.J. Barrow, and J.L. Adcock, Deakin University, Australia.

In this work we investigated the generation of products by 15-sLOX-1 under anaerobic conditions from a range of biologically significant LC-PUFAs including ARA, EPA, DHA and DPA. The use of highly unsaturated PUFAs, and also the radical scavenger 4-hydroxy-TEMPO, has provided greater insight into the peroxidase activity of 15-sLOX-1 through analysis of the regio-specificity of the products.

Anaerobic conditions were established by purging with N_2 and also by high substrate concentrations. Reaction conditions were also optimised to favour complete conversion of substrate. Products were isolated and characterised using NP- and RP-HPLC, ESI-TOF-MS, UV-visible and NMR spectroscopy. The major products of DHA found were 17-oxo-heptadecanoic acid, a novel C_5-DHA branched compound, and small amounts of 17-oxo-DHA stemming from the production of peroxyl and alkoxyl radicals. Analogous products have also been detected from omega-3 substrates EPA and DPAn-3, while omega-6 substrates ARA and DPAn-6 yield both oxodienes and ketodienes as major products. These compounds may have biological significance, in particular, 15-oxo-ARA has been shown to inhibit endothelial cell proliferation in cancer cell lines, and therefore is a potential pharmaceutical target for angiogenesis inhibition[1].

1. C. Wei, P. Zhu, S.J. Shah, I.A. Blair, *Mol. Pharmacol.* 76 (2009) 516-525.

6. **Enzymatic Biodiesel Synthesis of Palm Fatty Acid Distillate (PFAD) Using Blended Alcohol via Two-step Lipase-catalyzed Transesterification.** J.I. Ryu, N. Choi, and I.H. Kim, Korea University, Republic of Korea.

Biodiesel was synthesized successfully from palm fatty acid distillate (PFAD) and a blended alcohol via lipase-catalyzed transesterification. The blended alcohol, which was used as an acyl acceptor was prepared from methanol and 1-butanol. Lipozyme TL IM from *Thermomyces lanuginosus* was used as a biocatalyst and substrate molar ratio was 1:1 (PFAD to alcohol). The effects of the molar proportions of methanol in the blended alcohol, reaction temperature, and enzyme loading were studied as a function of reaction time. Among alcohols tested, 80mol% methanol in blended alcohol as a suitable acyl acceptor was selected. Optimum temperature and enzyme loading were 30^{o}C and 20% (based on the total substrate weight), respectively. The conversion of biodiesel was improved by addition of extra alcohol and dehydration using molecular sieve after a reaction time of 4h. As a result, a maximum conversion of ca. 96% was obtained under these optimized conditions.

7. **Substrate Selectivity of Novozym 435 in the Esterification of Glycerol with an Equimolar Mixture of Linoleic, Conjugated Linoleic, and Pinolenic Acids.** H. Woo[1], I.H. Kim[2], H.D. Choi[3], I.W. Choi[3], and B.H. Kim[1], [1]Chung-Ang University, Republic of Korea, [2]Korea University, Republic of Korea, [3]Korea Food Research Institute, Republic of Korea.

The selectivity of Novozym 435, an immobilized *Candida antarctica* lipase B toward linoleic (LA), conjugated linoleic (CLA), and pinolenic acids (PLA) was evaluated in the esterification of glycerol with an equimolar (27.3 mol% each) mixture of the fatty acids (FAs) to prepare triacylglycerols (TAGs) with

anti-obesity effects. The reaction was performed in a stirred batch reactor under the conditions (temperature, 30-70°C; reaction time, 1-24h; glycerol-to-FA mixture molar ratio, 1:3; enzyme loading, 10wt%; vacuum level, 0.4kPa). During the initial phase of the reaction, the order of esterification was PLA > CLA > LA at all temperatures investigated, whereas no significant difference in the esterification of the FAs was observed after the reaction reached the equilibrium. Novozym 435 showed the significant selectivity toward PLA over CLA and LA at the *sn*-2 position of TAGs throughout the entire reaction. The TAG content in the reaction products obtained at 70 °C and 24 h reached 98.9wt%. The resulting TAGs contained the same total contents (27.1mol% each) of PLA and CLA. However, PLA and CLA contents at the *sn*-2 position were 30.3 and 24.7mol%, respectively, indicating that the FAs were unevenly distributed throughout the glycerol backbone of the TAGs.

8. **Lipolytic Activity of Marine Actinomycetes.** T. Martins, C. Schinke*, and F.G.R. Reyes, University of Campinas, Brazil.

Lipases show a growing number of industrial applications (detergents, food industry, biodiesel biosynthesis, bioremediation). The industrial demand for different lipases is continuing to stimulate the screening and isolation of new lipolytic microorganisms. Marine bacteria are an unexploited source of lipases. We investigated the ability of 35 marine actinomycetes to produce lipases that hydrolize olive oil, soya biodiesel (methyl ester), candelilla and carnauba wax when grown for seven days on Rodamine B agar at 25-27°C. Fatty acids resulting from the hydrolysis form a complex with the dye, becoming fluorescent when visualized under U.V. light (366nm). All strains except one managed to grow on olive oil and biodiesel. Eighteen strains developed fluorescence on olive oil, seven with an intense halo. On biodiesel, of the 17 positive strains three presented strong fluorescence. Twenty seven strains grew on candelilla and carnauba wax. No fluorescence was seen on these media indicating that growth was supported by other wax constituents (*n*-alkanes, fatty alcohols, resins), not by wax esters themselves. In conclusion, marine actinomycetes produce lipases that act on different esters of fatty acids with possible use in industry. Their ability to grow on hydrocarbons and toxic compounds like resins show potential use in bioremediation.

9. **Dietary Effects of Conjugated Linoleic Triacylglycerols on Body Fat Accumulation and Blood Lipids in High-fat Diet-induced Obese Mice.** H. Woo[1], M.Y. Chung[2], I.H. Kim[3], H.D. Choi[2], I.W. Choi[2], and B.H. Kim[1], [1]Chung-Ang University, Republic of Korea, [2]Korea Food Research Institute, Republic of Korea, [3]Korea University, Republic of Korea.

This study examined the dietary effects of conjugated linoleic acid (CLA) in the form of triacylglycerols (TAGs) on body fat accumulation and blood lipid profiles in diet-induced obese mice. TAGs containing 70.3% CLA (CLA-TAGs) were prepared via a lipase-catalyzed esterification of glycerol with commercial CLA mixtures. Five-week-old male C57BL/6J mice (*n* = 13-14 per group) were fed normal diet (control), high-fat diet (HFD, 60% calories from fat), HFD plus 1% CLA-TAG (HFD-1% CLA), and HFD plus 2% CLA-TAG (HFD-2% CLA) for 12 weeks. Repeated measure ANOVA results showed that the body weight was significantly lower in HFD-2% CLA group than in HFD group between weeks 9-12, whereas no significant difference in the body weight was found between HFD-1% CLA and HFD groups throughout the entire period of feeding. After 12 weeks of feeding, HFD-2% CLA significantly reduced the epididymal and retroperitoneal fat weights and also significantly increased the liver weight compared to HFD. Serum triglyceride level was significantly lower in HFD-2% CLA group than in HFD group but serum total and LDL cholesterol levels of HFD-2% CLA group were significantly greater than those of HFD group.

10. **The Kinetics Mechanism of Enzymatic Synthesis of Phosphatidylserine in Different Reaction Systems.** Z.Q. Duan, H. Sun, and Y.L. Xue, Academy of State Administration of Grain, China.

We investigated the enzyme-mediated phosphatidylserine preparation in the eco-friendly solvents γ-valerolactone (GVL) and 2-methyltetrahydrofuran (2-MeTHF) previously, which possessed obvious advantages over the traditional processes in terms of high efficiency. Herein, the reaction mechanism in various systems was elucidated from the points of view of kinetics for the first time. The results indicated that the rate constants of the transphosphatidylation of PC with L-serine in GVL and 2-MeTHF were much bigger than the ones in the biphasic system and the purely aqueous system, while the rate constants of the reverse reaction were a little smaller, and thereby, the equilibrium constants were much higher which

resulted in higher PS yields. Additionally, the reaction temperature exerted a significant influence on the equilibrium constant and the highest value was achieved at 40°C in 2-MeTHF. This work can be considered as a useful reference for elucidating the enzymatic reaction mechanism using the tools of the chemical reaction engineering.

11. **A Process for Recovering Genistein 7-O-phosphate, a Water Soluble Alternative of Genistein, from Fermentation Broth.** W.Y. Lo and N.W. Su, National Taiwan University, Taiwan.

Genistein, one of the primary bioactive agents in soybeans, has a number of pharmacological and biological activities; however, low water solubility and poor bioavailability limit its use. Previous study revealed a water-soluble phosphate conjugate of genistein, genistein 7-O-phosphate (G7-P), generated by biotransformation of *Bacillus subtilis* var. natto BCRC 80517 with genistein. This study aimed to develop a feasible process for recovering G7-P from the fermentation broth of biotransformation. The effect factors including the harvested broth with various pHs for extraction, extraction efficiency of various solvents, and adsorption and desorption capability of various macroporous resins were investigated in this work. The results showed while the pH of the harvested broth was adjusted to 1, and then subjected to Dianion HP-20 resin, the most capacity for G7-P adsorption was approximately 38mg/g. After extensively washing with 5 bed-volume 1% aqueous NaCl, pH 1. The resins were eluted with 2 bed-volume 5% ethanol to remove impurities, and subsequently eluted with 4 bed-volume 30% ethanol. The G7-P concentrate could be obtained from 30% ethanol eluted fraction with 70% recovery and approximately 70% in purity.

12. **Functional Waxes Derived Plant Oils and Wax-nanocellulose Composites.** Y.S. Mugo, L. Huybregts, and C. Rusin, MacEwan University, Canada.

Waxes are long-chain hydrocarbon compounds with diverse applications in the food, cosmetic, pharmaceutical and chemical industries. The global market for waxes is expected to grow to $11.3 billion by 2015, with an increasing emphasis on renewable feedstocks and “green chemistry” as opposed to conventional petrochemical-based processes. In the presentation tailor made specialty hydroxylated biowaxes from Canola, Flax, Hemp and Camelina oils will be demonstrated. The development of the waxes technology entails deriving nearly 100% of the reagents needed in the transformation from the same oil crop biomass, ensuring maximum value addition, benign products and a sustainable process. Tribological and rheological properties of the waxes will be discussed. Key target applications of these waxes are as lubricants, cosmetics, printing inks, and waxed paper for food packaging, which will be discussed.

Using electrospray process, the hydroxylated biowaxes are converted to nanolipids which afford enhanced and unique tribological properties. Further, interaction of nanolipids and modified nanocellulose has been found to afford a unique class of composite materials, which will be demonstrated in the talk.

13. **Lipase-catalysed Regioselective Synthesis of Flavone *C*-glucosides Esters and High-efficiency Oil-soluble Antioxidant of Bamboo Leaves (eAOB-o).** L. Liu and Y. Zhang*, Zhejiang University, China.

AOB-w is a water-soluble antioxidant extracted from bamboo leaves, containing four flavone *C*-glucosides (orientin, isoorientin, vitexin, and isovitexin) as functional components. Flavone *C*-glucoside esters were firstly synthesized by three lipases (CALB, RM IM and TL IM) in organic solvent system or solvent-free system. Results indicated that C12 fatty acid ester could exhibited the optimal antioxidant capacity, CALB was the most efficient lipase in both systems, and higher conversion yields were achieved in solvent-free system. Highest conversion was obtained with 1% water content for CALB and 2% water content for RM IM and TL IM. All lipases displayed absolute 6"-regioselectiveties with monoester. It’s required to consider both of hydrophobicity and antioxidant capacity to prepare high-efficiency oil-soluble antioxidant. Regression equations were built up to illuminate the relationship between conversion yield of flavone *C*-glucoside and antioxidant capacity of its corresponding ester. Results indicated that highest antioxidant capacity could be exhibited when conversion yield was nearly 60% for flavone *C*-glucosides. Based on reaction parameters of flavone C-glucosides, high-efficiency eAOB-o was prepared, which could be expected as an excellent oil-soluble antioxidant in lipid system.

14. **Evaluation of a Promising Glycerol Derivative, D-glyceric Acid, as Cell Proliferating Agents and Protective Solutes.** S. Sato and H. Habe*, National Institute of Advanced Industrial Science and Technology (AIST), Japan.

In the oleochemical industrial fields and the process of producing biodiesel fuel (BDF) from plant oils, glycerol can be obtained at approximately 10 wt% as a by-product in the transesterification of triglycerides. The total amount of glycerol produced worldwide is estimated to be more than a million tons a year, resulting in a surplus of glycerol. Hence, glycerol is an attractive feedstock for producing useful chemicals. Recently, we have developed a technique to biotechnologically produce D-glyceric acid (GA) from glycerol with a yield of more than 100g/L in 6-d incubation, and have found some functionality of GA to expand its application. Here, we report the ability of less than 1% GA sodium salt (GA-Na) to activate the proliferation of human dermal fibroblasts by ~45%. Also, GA-Na showed the ability to protect biological molecules in vivo, i.e., the protective effects on DNA scission by hydroxyradical and protein aggregation by heat.

15. **Essential Fatty Acids from Microalgae for Food Application.** A. Matos[1], R. Feller[1], E. Moecke[1,2], J. Oliveira[1], A. Junior[1], R. Derner[1], and E. Santanna[1], [1]Federal University of Santa Catarina, Brazil, [2]South University of Santa Catarina, Brazil.

Algae can provide essential fatty acids, such as a-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, α-linolenic acid, and arachidonic acid.Typical western diet provides dramatically skewed ω-3/ ω-6 ratios toward ω-6, promoting the pathogenesis of many diseases. This is mainly due to the disproportionate consumption of ω6-rich vegetable oils (e.g. sunflower, peanut, corn) in detriment of seafood, nuts, and other sources of ω-3.

This study aimed to screening the lipid and fatty acids composition of six microalgae strains: *Chlorella vulgaris*, *Spirulina platensis*, *Nannochloropsis oculata*, *Nannochloropsis gaditana*, *Porphyridium cruentum,* and *Phaedactylum tricornutum*.

The lipid content ranged from 17.5 to 7.4%. *N. oculata* showed the highest lipid content 17.5%. *N. gaditana* and *S. platensis* showed lipid rich in saturated fatty acids, especially palmitic acid. Monounsaturated fatty acids were observed at high content in *N. oculata* (35.0%) and *P. tricornutum* (28.0%). High polyunsaturated fatty acids (PUFA's ω 3) contents were observed in *C. vulgaris* and *P. tricornutum.* Marine algae *P. tricornutum* and *P. cruentum* are also source of essential long chain PUFA's, mainly composed of eicosapentaenoic (20:5 ω 3) and arachidonic (20:4 ω 6) acids. *N. oculata* presented ω 3/ ω 6 ratio of about 7.2 - toward to ω 3.

16. **Towards a Yeast-based Multivalue Technology Platform.** L.A. Garay, I.R. Sitepu, H.E Teh, T. Cajka, L. Anderson, A.K. Franz, O. Fiehn, J.B. German, K.L. Boundy-Mills, and Z. Pan, University of California Davis, USA.

An important aspect in microbial biotechnology industrialization is economic feasibility. A common approach is to develop a microbial culture to produce a single product. This strategy in many cases fails to be economically feasible. Creating a portfolio of products out of a microbial culture can render the process economically attractive. This work utilized Rhodosporidium babjevae, a pink, oleaginous basidiomycete yeast sourced from the Phaff Yeast Culture Collection to test the concept at a lab scale. The yeast was cultured in shake flasks, harvested, and passed through a screw press to recover oil, and high protein yeast meal. The oil was further characterized to contain a triacylglycerol profile ideal for biofuel conversion or high heat cooking since it is rich in monounsaturated fatty acids. Pigments were analyzed using TLC, and could be further processed into high value products. Overall, this work establishes a framework to procure multiple high value products from yeast through an economically feasible, environmentally friendly and sustainable technological platform.

17. **Enzymatic Glycerolysis Under Supercritical CO_2 Conditions for Producing a Diacylglycerol (DAG)-enriched Oil.** N. Vafaei[1,2], M.G. Scanlon[1], P.J.H. Jones[1,2], C.B. Rempel[1,3], and N.A.M. Eskin[1], [1]University of Manitoba, Canada, [2]Richardson Center for Functional Food and Nutraceuticals, Canada, [3]Canola Council of Canada, Canada.

Diacylglycerol (DAG)-enriched oils were introduced because of their beneficial effect in reducing both body weight and the accumulation of visceral fat. Applying an environmentally friendly technology for producing DAG is the focus of this study. A supercritical CO_2 (SC-CO_2) system was developed for the enzymatic glycerolysis of a DAG-enriched oil from soybean oil in order to exclude the use of organic solvents. The reaction was conducted under SC-CO_2 and/or atmospheric conditions at 60°C, 75 bar using an anhydrous glycerol/soybean oil

at a molar ratio of 1:2 and novozyme 435, an immobilized lipase preparation on acrylic resin. A time course study of the enzymatic reaction for 1, 3, 4 and 8 hours was conducted. Nuclear magnetic resonance (NMR), specifically ^{31}P-NMR, was used to determine the DAG-enriched oil composition. A much higher yield of total DAG (49%) specifically 1,3-DAG, was obtained under SC-CO_2 conditions compared to the reaction conducted under atmosphere conditions (5.5%). Under SC-CO_2 conditions, the weight percentages for 1-MAG-primary were, 4.8-8.4%; 1-MAG- secondary, 4.7-8.6%; 2-MAG, 0.89-1.69%; 1,3-DAG, 25-31%; 1,2-DAG, 18-23%, and tocopherols, 0.93-1.11%. Irrespective of conducting the reactions under SC-CO_2 or non-supercritical conditions, the yield of 1,3-DAG was higher than 1,2-DAG.