BIO 1: Enzymatic Biodiesel

Chairs: H.C. Holm, Novozymes A/S, Denmark; and R. Burton, MARC-IV, Inc., USA

Technical Considerations for Biodiesel Production Using Enzyme Catalysis. R. Burton, MARC-IV, Inc., Pittsboro, NC, USA.

The search for alternative catalysts for the production of biodiesel has been of significant interest to industry.

One primary reason to replace conventional alkaline catalysis is to eliminate soap waste streams in commercial production. Furthermore, utilizing enzymes in the processing of fatty acid esters can eliminate waste water streams, enhance the co-product quality of glycerol, and provide the ability to use lower quality feedstocks. These low quality feedstocks like yellow grease with higher free fatty acid (FFA) content are largely underutilized for biodiesel due to the difficulty of processing these types of oils. This paper will evaluate the real world experiences of an enzymatic biodiesel plant. This operation has developed biodiesel production techniques using the immobilized enzymes Candida Antarctica Lipase B (CALB), as well as liquid enzyme formulations for the production of fatty acid esters.

A New Immobilized Lipase for Industrial Production of Biodiesel. S. Basheer, TransBiodiesel Ltd., Shfar am, Israel.

Lipases have been proposed since more than a decade for the production of fatty acid short-chain alkyl esters for use as biodiesel. Either esterification of free fatty acids and a short-chain alcohol or transesterification of glycerides and a short chain alcohol, typically methanol, have been predominantly applied for enzymatic production of biodiesel. Reported results showed that different formulations of various native lipases exhibited high degree of intolerance to methanol typically used in the esterification/transesterification reactions of different oil feedstocks to produce biodiesel. In addition to inhibition of the catalytic activity caused by methanol, the use of commercially available immobilized lipases for the production of biodiesel has faced severe mass transfer constraints. This work will present a new developed immobilized enzyme preparation characterized with its high activity for the catalysis of both esterification as well as transesterification reactions, simultaneously. Based on the developed enzymatic process different pilot model units have been designed and built for the production of biodiesel using high FFA's multiple feedstocks. The recent results from a commercial continuous unit for the enzymatic production of biodiesel using high FFA's multiple feedstocks will also be presented.

Enzyme Catalyzed Biodiesel Using Liquid Lipases. P.M. Nielsen, Novozymes A/S, Bagsvaerd, Denmark.

During the last year the enzymatic transesterification using liquid lipase (the BioFAME process) has been proven in full production scale in two plants in USA. The learnings from these plants are shared in this presentation in the discussion of how to control the process to get biodiesel from used cooking oil and DDGS corn oil. The most important parameters have been:

- Securing correct pretreatment to keep the enzyme stable
- Controlling the methanol addition rate
- Recover the heavy phase and re-use the enzyme
- Downstream processing

We will describe the details in controlling the reaction and document the BioFAME process. The robustness for tolerance of changes in FFA will be discussed, as well as the perspectives and how to operate with different types of oil qualities.

Effect of Phospholipids on Free Lipase-mediated Biodiesel Production. W. Du, Tsinghua University, Beijing, China.

Free lipase-catalyzed biodiesel has drawn more and more attentions in recent years because of its advantages of lower cost and faster reaction rate. Utilizing free lipase to convert low quality oils, such as crude vegetable oils and microbial oils, is beneficial to further reduce the cost of biodiesel production. However, these oils typically contain some amount of phospholipids. Phospholipids were found to affect the lipase-catalyzed process and further influence the enzyme's thermal stability in biodiesel production process. In this work, free lipase NS81006-mediated biodiesel production from oils containing phospholipids at varied temperature was investigated systematically. It was found that increasing temperature led to a decreased fatty acid methyl esters (FAME) yield and poor reuse stability of the lipase in converting oils containing phospholipids for biodiesel production. Further study showed that this inhibitory effect of

temperature was mainly attributed to the coexistence of phospholipids and methanol in the system. A novel two-step enzyme-mediated process was developed, with which the above-mentioned inhibitory effect was eliminated, and a FAME yield of 95.1% could be obtained with oils containing 10% phospholipids as the feedstock.

Large Scale, Enzyme-catalyzed Methyl Ester Manufacturing—Justification and Execution. B. Baughman, Blue Sun Biodiesel, Wheat Ridge, CO, USA.

Practical focus on the challenges facing investors/owners/engineers choosing to engage the Novozymes enzymatic catalyst in methyl ester manufacturing. Substance is based on decades of practical experience designing, building, operating and maintaining industrial manufacturing facilities in the oleo and renewable fuels sectors and the recent commercialization of the Novozymes enzyme catalyst at 30 mgy scale. Practical discussion of the six pillars of manufacturing: Regulatory Compliance Plan, Volume/Logistics Plan, Quality Plan, Capital Plan, Expense Plan, HR/HR Organizational Development Plan.

From Promise to Practice: Real-life Advances in Enzymatic Biodiesel. K.P. Staller, Patriarch Oleo, Inc., Garrett, IN, USA.

Enzymatic biodiesel was brought to a commercial reality this past year. Characteristically, the path to commercial operation turned out to be far more difficult than anticipated, but this presentation will show how the obstacles were overcome.

Utilization of Liquid Lipase on a Commercial Scale. R. Hobden, Viesel Fuel, LLC, Stuart, FL, USA.

In the transition for enzymatic biodiesel from lab/pilot scale to commercial scale, not only does the catalytic reaction need to be evaluated, but the supporting unit operations need also be optimized to make enzymatic biodiesel a real option for biodiesel production. Many of these processes are universal concerns for biodiesel producers, such as feedstock diversity and crude biodiesel refining to meet ASTM specs, but because of the sometimes subtle differences in the enzymatic reaction, unique solutions are required to address these concerns. Specifically, these unit operations include the recovery and reuse of the enzymes to catalyze multiple batches, the reduction of FFA from the crude biodiesel, and proving the use of the lowest quality feedstocks. In the research department at Viesel Fuels, each of these processes is at different stages of development, implementation, and optimization. The results presented offer great promise of making the large scale enzymatic biodiesel process both technically and commercially feasible.

Biodiesel Through Enzymatic Transesterification: The Ecological and Economical Alternative for Low Quality Oil Feedstocks. M. Kellens, Desmet Ballestra Group, Zaventem, Belgium.

The increasing tendency to use low quality oils as feedstock for biodiesel production has triggered the development of various chemical routes to make them suitable for the conventional, mostly alkaline based chemical transesterification process. High FFA oils are pre-esterified using strong acids, special resins, or bound with glycerine, after which they then undergo a further transesterification to convert all fatty acids into their methylester form.

The development of robust Lipase enzymes, able to attack fatty acids in both free and bound form, has made it possible to convert low quality high acidity feedstocks into biodiesel with minimum pretreatment.

The enzymatic process has been proven to work on used cooking oils, fatty acid distillates and high acidity oils like DGS corn oil. But the main question to answer is how to make the enzymatic process cost-efficient enough to compete with the other routes. Some comparisons are made and examples given to illustrate the true potential of the enzymatic process.

BIO 1.1/S&D 1.1: Merging Biology and Surfactants

Chairs: D.G. Hayes, University of Tennessee, USA; G.A. Smith, Huntsman Corp., USA; and D. Solaiman, USDA, ARS, ERRC, USA

Sophorolipids: Microbial Synthesis and Biotechnological Opportunities. I.N.A. Van Bogaert and W. Soetaert, Ghent University, Ghent, Belgium.

Sophorolipids are surfactants which are produced by specific yeast species, such as *Starmerella bombicola*. Thanks to the high yields of this biological process (over 400 g/L can be obtained) and the environmentally friendly character of these biosurfactants, they caught the attention of several academic institutes and later on companies. Indeed, sophorolipids are one of the few biosurfactants which are commercially produced and applied.

Although certain microbial biosurfactants are already find applications, their usage range could be further broadened if more structural variants would be available, in this way creating molecules with different physoco-chemical properties and functions. Yet, creating variation in a biological controlled process is not as easy as modifying a chemical reaction; rendering the creation of tailored biosurfactants quite a challenge.

We try to tackle this shortcoming by a combined fundamental and applied approach: getting insight into the biosynthetic pathway and its regulation, control and engineer it. In order to achieve this, we *de novo* sequenced the full genome of the yeast and set up extensive transcriptomics and proteomics experiments. We will discuss the identification of the sophorolipid core pathway and will give several examples of how it can be engineered to produce tailored glycolipids.

Identification of Long-chain Alcohol Oxidase AOX1 from *Starmerella bombicola* and Knocking Out the AOX1 Gene Leads to Improved Alkyl Polyglucoside Production. F. Takahashi, K. Igarashi, and H. Hagihara, Eco-Innovation Research, Kao Corp., Wakayama-shi, Wakayama, Japan.

Alkyl polyglucoside (APG) has good properties as a cleaner, foamer, and emulsifier, and does not hydrolyze at an alkaline pH. In addition to its advantages as a traditional alkylglucoside surfactant, APG is a low-irritant surfactant that is nontoxic and easily degradable in the environment. Thus, APG is considered an environmentally friendly surfactant. We have been working to develop new APG synthesis methods aimed at decreasing the cost as well as the burden on the environment and facility, and expanding the structure.

Starmerella bombicola glycosylates long-chain omega-hydroxy fatty acids and it also directly glycosylates secondary alcohols. Although it is difficult to directly glycosylate primary alcohols, they are easily converted to the corresponding fatty acid. To redirect unconventional substrates toward APG synthesis, the long-chain alcohol oxidation pathway was blocked at the genome level by knocking out the fatty alcohol oxidase gene. The total gene sequence of the *S. bombicola* AOX1 (2046 bp) was cloned. Knock-out mutants were evaluated by fermentation in 1-tetradecanol. The mutants produced much higher amounts of APG, indicating that the substrate had been redirected toward APG synthesis in those strains.

Incorporation of Membrane Proteins in the Bicontinuous Microemulsion Phase of a Winsor-III System. D.G. Hayes[2], R. Ye[2], V.S. Urban[1], S.V. Pingali[1], and H. O'Neill[1], [1]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]University of Tennessee, Knoxville, TN, USA.

Membrane proteins (*MPs*) account for approximately 33% of proteins encoded by the human genome, and are targets for 50-60% of therapeutical agents. They are difficult to characterize due to their poor solubility in aqueous media, and therefore account for only 3% of three-dimensional structures within the Protein Data Bank. We have successfully incorporated several different *MP*s (e.g., cytochrome c, melittin, phtotosystem II, and light harvesting complex I and II) Iinto the middle, bicontinuous microemulsion (Bicont ME), phase of Winsor-III systems at concentrations > 1 g/L, formed using Aerosol-OT / 1,3-dioxolane-based alkyl ethoxylate binary surfactant system. Unlike other surfactant self-assembly systems employed for MP solubilization, the Bicont ME phase is optically transparent, isotropic, and contains surfactant monolayers of near-zero curvature, to reduce denaturation. The MP-laden Bicont ME phases have been investigated by circular dichroism to analyze for perturbations in the MPs' secondary structure, and by small-angle neutron scattering (SANS) to determine structural perturbations of the phase's nanostructure. Via SANS we determined that the incorporation of MPs reduces the average diameter

of the Bicont MEs' nanochannels and increases the surface area per volume of the MEs.

Surfactants Based on Algae Oil. G.A. Smith and M. Coleman, Huntsman Corporation, The Woodlands, TX, 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 for food production. 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). Different 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.

Superior Characteristics of Biosurfactant Surfactin and Its Application Potentiality. S. Yanagisawa[1], T. Nagano[1], M. Izumida[1], T. Imura[2], T. Taira[2], and D. Kitamoto[2], [1]New Business Development Division, Kaneka Corporation, Osaka, Japan, [2]Research Institute for Innovation in Sustainable Chemistry, National Institute for Advanced Science and Technology, Tsukuba, Japan.

Surfactin (SF) is one of the most promising biosurfactants produced by fermentation under mild conditions with naturally derived raw materials and a type of safe microorganism, *Bacillus subtilis*. It is produced and currently commercially available as sodium salt (Kaneka Corporation). This sustainable product is both human- and environment-friendly. Results of skin irritation test show that it is much milder than other typical surfactants. In fact, it showed no observable skin irritation at generally used concentrations. Moreover, it was degraded almost completely within a week in the biodegradation test.

The structure of SF is unique compared to that of other surfactants. SF has a heptapeptide head group interlinked with a ß-hydroxy fatty acid. Due to its unique cyclic peptide structure, it shows excellent surface and self-assembling properties. It exhibited a dominantly low CMC (critical micelle concentration) value of 0.0003%. It was also found that the molecules spontaneously formed giant aggregations in aqueous solution by freeze-fracture electron microscopy (FFEM). Undoubtedly, it has many interesting potential applications in a variety of fields such as cosmetics and homecare industries.

Production and Antimicrobial Property of Glycolipid Biosurfactants. D.K.Y. Solaiman, R.D. Ashby, and J.A. Zerkowski, USDA, ARS, ERRC, Wyndmoor, PA, USA.

Microbial glycolipids such as rhamnolipid (RL) and sophorolipid (SL) are an important class of biosurfactants with excellent surface tension-lowering activity. Besides their surfactant- and environment-friendly properties, however, additional value-added property such as bacteriocidal activity is needed to further heighten their commercial interests. We devised a straightforward production scheme and HPLC analysis method for synthesis and characterization of monorhamnolipid (R_1L) and sophorolipid (SL) produced from patent-strain *Pseudomonas chlororaphis* NRRL B-30761 bacterium and *Rhodotorula bogoriensis* yeast, respectively. R_1L and SL were then incorporated into biodegradable poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) films. Tests on acne-causing bacterium (i.e., *Propionibacterium acnes*) and on tomato-rotting causative agent (i.e., *Pseudomonas corrugate*) showed that these glycolipids possessed excellent antimicrobial activity. The finding adds to the antimicrobial spectrum of RL and SL, thus further increasing the potential commercial values of glycolipid biosurfactants.

Production of Rhamnolipids from Unique Pseudomonads. G. Deluga, D. Derr, N. Lohitharn, D. MacEachran, J. Ulrich, W. Jordan, and R. Mirani, Logos Technologies, Ashburn, VA, USA.

The promise of biosurfactants has a long and storied history of having commercial potential (1). One of the major classes of biosurfactants is glycolipids that have the potential to replace a number of petroleum based surfactants due their sulfate and phosphate free nature while having superior surfactant properties. This class of glycolipids are characterized by either a mono or di rhamnose head group and a 3-(hydroxyalkanoyloxy)alkanoic acid tail that varies in molecular weight according to a number of factors including feedstock, fermentation time and

Pseudomonas strain. We have developed three strains of Pseudomonads that produce rhamnolipids at high titers.

This presentation will discuss the three strains of Pseudomonads that have been isolated for the overproduction of both mono- and di-rhamnolipids. Each strain has a unique rhamnolipid molecular weight profile for a specific feedstock. Results will be presented discussing how these two variables interact. Data demonstrating a correlation between the molecular weight distribution of the rhamnolipid isolates and the CMC will be presented. These biosurfactants have the characteristics and can meet the cost targets necessary for large scale commercialization.

(1) Desai JD, Banat IM (March 1997). Microbiol. Mol. Biol. Rev. 61 (1): 47–64.

Biosynthesis of Surface-active Lipids. M. Falkeborg *(Honored Student Award Winner and The Manuchehr Eijadi Award Winner),* R. Gao, S. Song, L.Z. Cheong, X. Xu, and Z. Guo, Dept. of Engineering, Aarhus University, Aarhus, Denmark.

Surface-active lipids find applications in food and pharmaceutical industries and the interest for their economically feasible and ecologically friendly production and derivatization is increasing. This presentation summarizes our recent progress in 1) production of biosurfactants *via* high-cell density fermentation; and 2) enzymatic synthesis of phosphatidyl saccharides for preparation of nanoliposomes with enhanced stability.

Sophorolipids have received special attention due to their biodegradability, low toxicity, and production from renewable resources. To produce sophorolipids with high time-space efficiency, a high-cell-density fermentation strategy was applied. The nutrient concentration and physical parameters were optimized for enhanced volumetric productivity. The protocol was implemented in a 10-L fermentor and a remarkably high volumetric productivity (>200g isolated sophorolipids/L/day) was achieved.

Derivatization can tailor surface-active lipids to specific applications. Phospholipids are known to form liposomes capable of encapsulating sensitive ingredients; however, drying processes are usually required and cryoprotectants are needed to stabilize the liposomes. To overcome this, an enzymatic method for incorporating sugar moieties into phospholipids was developed. The resulting phosphatidyl saccharides were capable of forming liposomes with increased stability during dehydration.

BIO 2: Biocatalysis I

Chairs: C.T. Hou, USDA, ARS, NCAUR, USA; and T. Nagao, Osaka Municipal Technical Research Institute, Japan

Extensive Fatty Acid Specificity Analysis of Various Lipases on Hydrolysis Using Randomized Triacylglycerol Prepared with Wide Range Variation of Fatty Acids as Substrate. T. Nagao, S. Tanaka, and H. Nakano, Osaka Municipal Technical Research Institute, Osaka, Japan.

Lipase has been used as a catalyst in many purposes, such as production of PUFA-rich oils and purification of PUFAs. Lipases possess several substrate specificities, and among these specificities, fatty acid specificity is the most important property for practical applications. We thus evaluated fatty acid specificity of 11 commercial available lipases on their initial hydrolysis reaction using two random esterified triacylglycerols prepared with wide range variation of fatty acids (fatty acids from C4 to C18, and fatty acids from C14 to C24 including PUFAs) as substrates.

Candida rugosa lipase showed weak activity against C4:0, C6:0, GLA, and fatty acids composed of C20 and >C20. *Rhizomucor miehei* and two *Rhizopos* lipases acted effectively against fatty acids composed of C20 and >C20, but not against C4:0, GLA, and DHA. *Pseudozyma antarctica* (re-classified from *Candida antarctica*) lipases A and B showed strong activity against wide range fatty acids including PUFAs except long chain saturated fatty acids. Fatty acid specificity of *Penicillium roqueforti*, *Alcaligenes* sp., *Burkholderia cepacia*, *Pseudomonas aeruginosa*, and pancreatic lipases were also evaluated.

Synthesis of Polyglycerin Fatty Acid Esters by Lipase Reactions. Y. Nishiyama, Y. Kataoka, H. Uehara, and Y. Ueda, The Nisshin OilliO Group, Ltd., Yokosuka, Kanagawa, Japan.

Polyglycerin fatty acid ester (PGE) has various features depending on its degree of esterification. Generally, PGE available in the market is made by chemical synthesis methods, and it is known that the number of fatty acids esterified to a polyglycerin molecule varies around some central value. On the other hand, PGE can be synthesized also by lipase reactions, so we may be able to synthesize PGE whose degree of esterification is narrowly distributed via the lipase's selectivity.

First, we carried out the screening of lipases which could synthesize PGE from polyglycerins and fatty acids. Then we found that some lipases had the capability to synthesize PGE even in organic solvents, and in particular, lipase OF (*Candida cylindracea*) could synthesize monoester with high selectivity in *tert*-butyl alcohol. We next tried to optimize some reaction conditions to increase the reaction rate. Then we found that initial water content in the reaction system significantly affected the reaction rate. Consequently, we were able to acquire the high-purity polyglycerin fatty acid monoester in a relatively short reaction time.

Production of Biodiesel from Acid Oil, a Low Value Feedstock via a Two-step Enzymatic Transesterification. N. Choi[1,2], J. Baik[1,2], S.W. Yoon[1,2], N.H. Kim[1,2], and I.H. Kim[1,2], [1]Dept. of Food & Nutrition, Korea University, Seoul, Republic of Korea, [2]Dept. of Public Health Sciences, Graduate School, Korea University, Seoul, Republic of Korea.

A two-step enzymatic transesterification in a solvent-free system was attempted as a novel approach to produce biodiesel using two different lipases. This study involved an employment of a low value feedstock and an appropriate combination of two different lipases. Acid oil prepared from rice bran oil soapstock and ethanol as a renewable acyl acceptor were used as substrates. Three immobilized lipases, namely Novozym 435 from *Candida antarctica*, Lipozyme RM IM from *Rhizomucor miehei*, and Lipozyme TL IM from *Thermomyces lanuginose* were employed as biocatalysts. Firstly, the optimum conditions for the three lipases were determined to be 1:5 of the molar ratio (acid oil to thanol), 30-40°C for the temperature, and 5-10% for the enzyme loading. A two-step transesterification was carried out under the optimum conditions of individual lipases obtained. For the order of using lipases in this two-step reaction, high cost lipases such as Novozym 435 or Lipozyme RM IM were employed at the first step for an extremely short reaction time (ca. 15 min). Subsequently, the remaining reaction was carried out using Lipozyme TL IM as a low cost lipase. In conclusion, more than 90% yield of biodiesel was obtained successfully within only 8 h of reaction time via the two-step lipase-catalyzed transesterification.

Genetic Engineering of Oil Palm: Challenges and the Way Forward. G.K.A. Parveez, A.R. Omar, M.Y. Abdul Masani, I.A.M. Dayang, B. Bahariah, S.M. Masura,

N.A. Hanin, W.N.W.S. Syuhada, U.S. Ramli, A. Othman, A.M. Muhammad Arif, I. Zamzuri, A.H. Tarnizi, A. Kushairi, S. Ravigadevi, Malaysian Palm Oil Board, Persiaran Institusi, Kajang, Selangor, Malaysia.

Genetic engineering with all the promises and advantages was considered and implemented almost 20 years ago with the objective of keeping the palm oil industry remains competitive. Successful production of transgenic oil palm using various methods and selection systems in addition to the isolation of useful genes and tissue-specific and constitutive promoters have push forward the genetic engineering programme towards producing novel and higher quality palm oil. 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. In order to produce the above targeted products using the isolated genes and promoters, constructions of various combinations of transformation vectors carrying different genes and promoters for specific targets have been successfully carried out.

Transformation of oil palm embryogenic calli with the above constructs has been successfully carried out and resulted in a number of transgenic palms that have been transferred onto soil in a contained greenhouse. Some of the challenges faced during the research and potential solution will be elaborated.

Production of Soy Polyol Oils: Results of Microbial Screening and Identification of Positive Cultures. C.T. Hou[1], D.P. Labeda[2], and K. Ray[1], [1]RPT, NCAUR, USDA, Peoria, IL, USA, [2]BFP, NCAUR, USDA, Peoria, IL, 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. Of the 650 cultures screened, 50 were active in converting soybean oil to polyol oil and DAGs products. The 11 most active strains were identified based on the sequences determined for their 16S rRNA genes. Two strains were identified as *Acinetobacter haemolyticus.* The rest of the strains belong to the genus *Pseudomonas*.

Enhancing the Thermostability of Engineered Phospholipase D. Y. Iwasaki, J. Damjanovic, and H. Nakano, Nagoya University, Nagoya, Aichi, Japan.

Transphosphatidylation catalyzed by phospholipase D (PLD) can be applied for the synthesis of various phospholipids. Wild-type *Streptomyces* PLD could not synthesize phosphatidylinositol (PI), but our recent studies have created mutant PLD having PI-synthesizing activity. The purpose of this study is to enhance the thermostability of the PI-synthesizing PLD for better performance in the PI synthesis.

We first employed combinatorial mutagenesis followed by high-throughput screening. Random mutations were introduced into selected positions having high B-factor. Screening of the libraries under restricted conditions yielded several single-point mutants. Combining these point mutations yielded double and triple mutants; one of which, D40H/T291Y was revealed to be the most efficient in the PI synthesis at high temperature.

Next, we employed rational design approach. The residue D40 is part of a nine-residue surface loop, which is solvent-exposed and highly fluctuating. Thus, we tried to enhance the stability by removing this unstable loop. Mutant enzymes lacking the loop showed better thermostability; the best variant showed 11.7 times longer activity half-life at 70°C than that of the parent. The deletion variants showed twofold higher PI yield in high-temperature PI synthesis, while at the same time produced less of the hydrolytic side-product, phosphatidic acid.

Liposomes as Drug Delivery Carrier for Manganese Porphyrin-based Anticancer Drug. T. Aikawa[1,2], S. Ito[1], T. Kondo[1,2], and M. Yuasa[1,2], [1]Dept. of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, Noda, Chiba, Japan, [2]Research Institute for Science and Technology, Tokyo University of Science, Noda, Chiba, Japan.

Our group has developed manganese porphyrin derivatives as an anticancer drug based on anti-oxidation effect, which can eliminate and exploit reactive oxygen species generated in cancer cell. To effectively accumulate the manganese porphyrin-based anticancer drug into targeted tumor, we prepared liposome-based drug carrier that can incorporate the porphyrins in its bilayer membrane.

The liposome composed of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) conjugated with transferrins(Trfs). Since receptors of Trf are overexpressed in cancer cell, the liposomes modified with Trf are expected to accumulate specifically in cancer cell. Size of the liposomes prepared by conventional sonication method was ~100 nm. Their surface charge was approximately zero (-0.2 –+0.4 mV). Flow cytometry analysis clearly showed that hepatic cancer cells tended to uptake the liposomes modified with Trfs compared to those without Trfs. In addition, confocal microscopy revealed that the liposomes were uptaken through endocytosis. In vivo analysis, the liposome modified with Trf significantly reduced the volume of tumor transplanted in mice compared to liposomes without Trf. The liposome system in this study is expected to enhance efficacy of incorporated drugs without side effects.

Poly(3-hydroxybutyrate) Synthesis from Crude Glycerine: The Methanol Effect. R.D. Ashby, D.K.Y. Solaiman, and G.D. Strahan, USDA, ARS, ERRC, Wyndmoor, PA, USA.

Polyhydroxyalkanoates (PHAs; bacterial polyesters) can be synthesized from refined and crude glycerine under suitable fermentation conditions. The composition of crude glycerine generally varies based on reaction efficiency and product recovery but usually contains (in addition to glycerine), small amounts of partial glycerides, FFAs, water and residual short-chain alcohol (usually MeOH). *Pseudomonas oleovorans* NRRL B-14682 produces poly(3-hydroxybutyrate) (PHB) from glycerine however; the concentration of MeOH present in crude glycerine-based fermentations has a reductive effect on PHB molecular weights. We have formulated a series of glycerine-based fermentations containing MeOH (conc. from 0 to 1 wt%) and characterized the properties of the resulting PHB polymers. ^{1}H NMR revealed that MeOH acts as a chain terminating agent causing a premature halt to polymer elongation. Diffusion ordered spectroscopy (DOSY) revealed decreasing diffusion coefficients in PHB polymers derived from fermentations containing higher MeOH contents demonstrating a reduction in PHB molecular weights. While new uses for crude glycerine continue to generate interest, it is important to understand the effect of all molecules present in the crude glycerine on the final products. This discussion will focus on the effects of methanol on the fermentation-derived PHB biopolymers

Novel Strategy for Lipase-catalyzed Synthesis of Biodiesel Using Blended Alcohol as an Acyl Acceptor. T.T. Zhao[1,2], D.S. No[1,2], and I.H. Kim*[1,2], [1]Dept. of Food & Nutrition, Korea University, Seoul, Republic of Korea, [2]Dept. of Public Health Sciences, Graduate School, Korea University, Seoul, Republic of Korea.

As a novel strategy, blends of methanol and ethanol were used as acyl acceptors for biodiesel synthesis from soybean oil by lipase-catalyzed transesterification. Based on enzyme screening, Novozym 435 from *Candida antarctica* and Lipozyme TL IM from *Thermomyces lanuginosa* were selected for the reaction. For optimization of the reaction, the effects of the molar proportion of methanol in the blends, temperature, and enzyme loading were studied. The two enzymes displayed similar reaction trends in the molar proportion of methanol in the blends. When temperature was increased from 30 to 50°C, no significant different in the yield of biodiesel was observed with Novozym 435, whereas there was a rapid decrease in the yield of biodiesel with Lipozyme TL IM. For enzyme loading, Novozym 435 gave the highest yield of biodiesel (=95%) with 5 - 10% (based on the weight of substrate). Meanwhile, Lipozyme TL IM showed a significant increase in the yield of biodiesel with higher enzyme loadings and the highest yield of biodiesel (=95%) was obtained when enzyme loading was over 15%. Additionally, the relative consumption rates of methanol and ethanol during the transesterification using the blends were investigated. For both enzymes, the reactivity of methanol was significantly higher than that of ethanol.

BIO 3: Biocatalysis II

Chairs: S.H. Yoon, KFRI, Korea; and J. Ogawa, Kyoto University, Japan

Application of Cytochrome P450BM-3 for the Conversion of Eicosapentaenoic Acid to Its Epoxide Derivatives. J. Ogawa[1], C. Ishikawa[1], T. Kimura[2], S. Kishino[1,2], and M. Hibi[2], [1]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan, [2]Laboratory of Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan.

We screened P450BM-3 mutants for conversion of eicosapentaenoic acid (EPA) under aerobic condition with NADPH. Through the screening, some mutants were found to produce several products (UK1, 2, 3, 4). Then, these products were purified and identified with LC-MS, NMR and GC-MS. Finally, these products were identified: UK1 was 14,15:17,18-diepoxy-eicosatrienoic acid (14,15:17,18-DEpETr), UK2 was 17,18-epoxy-eicosatetraenoic acid (17,18-EpETe), UK3 was 14,15-epoxy-eicosatetraenoic acid (14,15-EpETe), UK4 was 11,12-epoxy-eicosatetraenoic acid (11,12-EpETe). The reaction conditions were optimized with P450BM-3 mutants, and under the optimized conditions, mutant Al4_Ile converted 0.5 mg/ml EPA to 0.20 mg/ml 11,12-EpETe (conversion rate: 38.0% mol/mol). Mutant F87A converted 0.5 mg/ml EPA to 0.19 mg/ml 14,15-EpETe (conversion rate: 36.1% mol/mol). Wild type P450BM-3 converted 0.5 mg/ml EPA to 0.38 mg/ml 17,18-EpETe (conversion rate: 72.2% mol/mol). Mutant L7V converted 0.5 mg/ml EPA to 0.075 mg/ml 14,15:17,18-DEpETr (conversion rate: 13.5% mol/mol).

Production and Application of Biofunctional Lipids. S.H. Yoon, Korea Food Research Institute, Seongnam-si, Kyunggi-Do, Korea.

Stereospecific analysis of fatty acid distribution of perilla oil was carried out. Linolenic acid was the major fatty acid in the sn-1, 2 and 3 positions. SC-CO2 extraction at 420 bar and 50°C and hexane extraction showed significantly higher oil yield than mechanical press extraction. Fatty acid compositions in the oils were virtually identical regardless of the extraction methods. SC-CO2-extracted perilla oils contained significantly higher contents of tocopherols, sterols, and policosanols than the mechanical press-extracted and hexane-extracted oils. Superoxide anion scavenging activities of ME of perilla, sesame and sunflower oil tested at 1 mg/mL concentration were 21.10, 13.25 and 3.14%, respectively. Hydroxyl radical scavenging activities of those samples tested at 1mg/mL concentration were 86.08, 93.30 and 93.17%, respectively. Refining process seemed to remove the phenolic compound during oil processing. Antiradical substances in perilla and sesame oils responsible for scavenging DPPH radicals were present in the methanol fraction, while the antiradical substances in the sunflower oil were in the lipid fraction. DPPH scavenging activity of ME of sesame oil was significantly higher than that of perilla oil. Superoxide anion scavenging capacity of ME of perilla oils was found to be greater than that of both sesame and sunflower oils.

Manipulation of Lipid Synthesis in the Hydrocarbon-producing Green Alga *Botryococcus braunii,* a Potential Biodiesel Producer. I.A. Guschina and J.L. Harwood*, Cardiff University, Cardiff, Wales, UK.

Botryococcus braunii, a green colonial microalga, is an unusually rich renewable source of hydrocarbons (ranging from 15 to 75% of dry weight). Three distinct races of *B. braunii* have been described (A, B, and L) based on the hydrocarbons accumulated. Such organisms are believed generally to be similar to those giving rise to the world's petrochemical deposits.

A better understanding of the metabolic reactions involved in lipid biosynthesis in *B. braunii* is needed in order to manipulate and direct carbon fluxes into increased hydrocarbon production. For this we have used radiolabelled precursors such as ^{14}C-acetate, ^{14}C-glycerol and ^{14}C-glucose and measured their incorporation into polar glycerolipids, non-polar triacylglycerols and hydrocarbons. These lipid fractions were separated into individual classes and radio-incorporation quantified. A series of compounds were then used to manipulate key reactions involved in the biosynthesis. Both specific and non-specific effects were shown and their consequences will be discussed. These data provide important information on lipid accumulation in *B. braunii* and, as such, will be very useful for lipid manipulation and metabolic engineering of this potential industrial alga.

Utilization of Seaweed Biomass for Functional Lipid Production. K. Arafiles, Y. Eramoto, H. Iwasaka, Y.

Okamura, Y. Matsumura, Y. Nakashimada, and T. Aki*, Hiroshima University, Higashi-Hiroshima, Japan.

Marine biomass has been paid attention to as a sustainable resource for the production of renewable energy and value-added materials. We aimed at producing functional lipids such as polyunsaturated fatty acids, xanthophylls, and terpenoids from seaweed saccharides by using marine protists, thraustochytrids, as biocatalyst. Assimilation test revealed however that thraustochytrid strains from the genus *Aurantiochytrium* do not have ability to directly utilize such algal saccharides for their growth. Thus, a number of bacteria and fungi that can degrade and convert algal saccharides into suitable substrates for *Aurantiochytrium* were obtained from culture collection or isolated from natural environment, to serve as intermediate biocatalysts. By cultivating in media composed of culture supernatant of some algal saccharide-assimilating bacteria, the *Aurantiochytrium* strains were able to propagate and accumulate the target lipids. Detailed analysis on the fermentation profile will be demonstrated.

Storage Lipid Accumulation by Acyltransferase Mutants of *Yarrowia lipolytica*. M. Certik[1], P. Gajdos[1], T. Rossignol[2], and J.M. Nicaud[2], [1]Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic, [2]INRA UMR1319 Micalis, Jouy-en-Josas, France.

The yeast *Yarrowia lipolytica* features active "oleaginous" metabolic pathways that efficiently accumulate storage lipids mainly in the form of triacylglycerols (TAG) and sterol esters (SE). The final step in TAG synthesis is catalyzed by acyltransferases Dga1p, Dga2p and Lro1p, whereas Are1p is involved to SE biosynthesis. To study contribution of these acyltransferases in lipid storage, mutants of *Y. lipolytica* Q4-*LRO1yl*, Q4-*DGA2yl*, Q4-*DGA1yl* and Q4-ARE*1yl* were prepared by reintroduction of single acyltransferase gene under constitutive promoter into the Q4 strain (deleted in *LRO1yl*, *DGA2yl*, *DGA1yl* and ARE*1yl* genes). Mutants Q4 and Q4-ARE*1yl* were unable to form neither TAG/SE nor lipid bodies (LB), other mutants stored TAGs in LB with diverse fatty acid profiles. Strains Q4-*DGA2yl* and Q4-*DGA1yl* were the most efficient in TAG formation (approx. 2-3 times more than Q4-*LRO1yl*). Increased copy number of either *DGA2yl* or *DGA1yl* resulted in significant elevation of lipid content in cells with slight change of fatty acid profile. However, yeasts expressing *DGA1yl* formed several small LB, while *DGA2yl* expressing strains had tendency to form one or two large LBs. Similar acyltransferase mutants with deleted MFE gene (coding key enzyme involved to beta-oxidation) and SUC2 (invertase gene) have also been constructed.

The work was supported by grants VEGA 1/0975/12 and APVV-0662-11.

Enhanced Antimicrobial Activity of Monoglyceride of 7,10-dihydroxy-8(E)-octadecenoic Acid. H.R. Kim[1], C. Dasagrandhi[2], H.R. Son[1], F. Wang[1], and C.T. Hou[2], [1]School of Food Science and Biotechnology, Kyungpook National University, Daegu, Korea, [2]Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL, USA.

Structural modification of lipids via chemical reaction or microbial bioconversion can change their properties or even create novel functionalities. Enzymatic oxidation of lipids can be led to formation of oxylipin such as hydroxy fatty acids. One of the multi-hydroxy fatty acids, 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) was produced from oleic acid and lipid containing oleic acid by a bacterial strain *Pseudomonas aeruginosa* PR3. Recently we have shown that DOD presented strong antimicrobial activity against broad range of pathogenic bacteria. In this study, we tried to enhance the antimicrobial activity of DOD by formation of monoacylglyceride of DOD(DO-MG). DOD-MG was successfully produced with over 80% production yield by lipase-catalyzed esterification under the optimized reaction condition. Antimicrobial activity of DOD-MG was highly enhanced by ten times compared to that of DOD.

Enzymatic Treatment of Soybean Meal. A. Al Loman, Q. Li, N.V. Callow, S.M.M. Islam, and L.K. Ju*, University of Akron, Akron, OH, USA.

Soybean meal, on dry basis, contains about 50% proteins and 30-35% carbohydrates, the latter include soluble and insoluble carbohydrates of various molecular weights and compositions. We have been developing an enzyme-based approach for treating soybean meal and separating protein from carbohydrate. We selected strains and produced the optimal enzyme mixtures for the target purpose. The process conditions have also been evaluated and kinetic models developed. High protein recovery and enrichment are achieved and carbohydrate separated is explored for further value-added uses. Results of this work will be presented and discussed.

Immobilization of Phospholipase A1 on Polystyrene Resin and Its Application for Production of Diacylglycerols Through Glycerolysis of Soybean Oil.
Y. Wang[1], N. Liu[2], Z. Zhang[1], W. Bai[1], and M. Liu[1], [1]Dept. of Food Science and Engineering, Jinan University, Guangzhou, Guangdong, China, [2]College of Life Science and Engineering, Shanxi University of Science and Technology, Xi'an, Shanxi, China.

To overcome high cost of some commercial immobilized lipases in production of structured lipids, a phospholipase A1, was immobilized by adsorption and the immobilized phospholipase A1 was employed to catalyze a glycerolysis reaction of soybean oil to prepare diacylglycerols (DAG). Firstly, the effect of nature of the polystyrene supports, the kinetic behavior and stability of immobilized phospholipase A1 were evaluated. Six macroporous resins has been investigated as the supports. The adsorption process was modeled by Langmuir and Freundlich equations, and the experimental data was better fit for the former one. Combined strategies of scanning electron micrograph (SEM), thermogravimetric analysis (TGA) and fourier transform infrared (FTIR) spectroscopy were employed to characterize the immobilized phospholipase A1. The immobilized phospholipase A1 was used as the catalyst for DAG production through glycerolysis from soybean oil. In a solvent-free system, with a reaction of 12 h under the optimum conditions, an upper lipid layer of the reaction mixture with 53.7 wt% DAG content was obtained. The immobilized phospholipase A1 demonstrated a remarkable reusability in recycling experiments by reusing the recovered immobilized phospholipase A1 for 28 consecutive batches.

One-stage and Two-stage Enzymatic Syntheses of Structured Lipids from Extra Virgin Olive Oil for Use as Infant Formula Fat Analogs.
G. Pande[1], J.S.M. Sabir[2], N.A. Baeshen[2], and C.C. Akoh[1,2], [1]University of Georgia, Athens, GA, USA, [2]King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Structured lipids (SLs) with high palmitic acid content at the *sn*-2 position enriched with arachidonic acid (ARA) and docosahexaenoic acid (DHA) were produced using extra virgin olive oil, tripalmitin, ARA and DHA single cell oils free fatty acids. Four types of SLs were synthesized using immobilized lipases from *Candida antarctica* and *Thermomyces lanuginosus*, based on one-stage (one-pot) and two-stage (sequential) syntheses. The SLs were characterized for fatty acid profile, triacylglycerol (TAG) molecular species, melting and crystallization profiles, tocopherols, and phenolic compounds. All the SLs had >50 mol% palmitic acid at the *sn*-2 position. The predominant TAGs in all SLs were PPO and OPO. The total tocopherol content of SL2-1, SL2-2, SL1-1, and SL1-2 were 70.46, 68.79, 79.64, and 79.31 µg/g, respectively. SL2-2 had the highest melting completion (42.0°C) and crystallization onset (27.6°C) temperatures. The lipases had better reusability in two-stage synthesis but one-stage synthesis was a faster reaction and also resulted in higher ARA and DHA content than two-stage synthesis. All the SLs produced in this study may be suitable as infant formula fat analogs.

BIO 4: Biotechnology for Oilseed Improvement

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

Bringing Back the Castor Plant as a Domestic Crop. T.A. McKeon[1], X. He[1], D.L Auld[2], and S. Leviatov[3], [1]USDA-ARS WRRC, Albany, CA, USA, [2]Texas Tech University, Lubbock, TX, USA, [3]Evofuel Ltd., Rehovot, Israel.

The castor plant *Ricinus communis* L. produces castor oil, an important chemical feedstock used for manufacture of lubricants, detergents, plasticizers, polymers, paints and even chocolate. Because of the presence of the toxic protein ricin, and the expense of energy needed to detoxify castor seed meal, castor oil production is limited to a few exporting countries. Its cost is generally two to three times the cost of soy oil, even though it can be produced as cheaply. A low or no-ricin castor crop would support increased cultivation of castor. We have applied a sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of ricin in castor beans and screened a collection of castor breeding lines as well as several commercial varieties using this assay. Although the average content derived by extracting several seeds provided a fair representation of the ricin content for a given selection, there was enough seed-to-seed variation to justify determining the ricin content of individual seeds in order to select the lowest ricin producers. Ricin levels observed ranged from 1.16% to 6.25% by weight. We have also addressed concerns about castor as an invasive weed, and demonstrated that glyphosate is an effective means for eliminating even maturing castor plants.

Designing of a Prototype Soybean-based Feedstock for Aquaculture. T. Clemente, University of Nebraska-Lincoln, Lincoln, NE, USA.

The aquaculture industry is expected to expand over the coming years as a means to meet the growing demand for seafood. A challenge facing this expected expansion in aquaculture is meeting the aquafeed supply needs in a sustainable fashion that does not compromise the world's wild fisheries. An avenue to address this challenge is designing agquafeed formulations in which marine-based ingredients are replaced with land-based protein and lipid sources. To this end, we have formulated a feed for the high-quality finfish *Seriola rivoliana*, with a soybean protein concentrate inclusion level of 40%, and half of the lipid component sourced from a high omega-3 fatty acid soybean oil enriched with linolenic acid and high in stearidonic acid. We have further developed the oil component of this high omega-3 fatty acid soybean by creating a set of gene-stacks in the crop that leads to the simultaneous accumulation of the very-long chain omega-3 fatty acid, eicosapentaenoic acid (EPA), and the carotenoid, astaxanthin. Greenhouse and small-scale field trials conducted with the soybean events producing this novel oil revealed the levels of EPA and astaxanthin were sustained over generations, under both controlled and field conditions, with EPA levels reaching approximately 4% and astaxanthin accumulating to 25 µg per gram seed.

Helping Industry Turn Over a New Leaf: Game-changing Technology Enabling Oil Production in Leaves. A.G. Green *(AAOCS Lipid Research Award Winner)*, T. Vanhercke, J.R. Petrie, and S.P. Singh, CSIRO Plant Industry, Canberra, ACT, Australia.

Plant triglyceride oils offer great technical potential to provide much-needed renewable replacements to petroleum derived fuels and chemicals. However to expand plant oil production sufficiently to make major inroads into the high global demands for these industrial products will require game-changing technologies. A particularly promising recent development has been the metabolic engineering of leaves to synthesise and accumulate commercially significant levels of storage oils. Here we report that the coexpression of three genes involved in different aspects of TAG production resulted in the accumulation of more than 15% TAG (17% total lipids) by dry weight in *Nicotiana tabacum* (tobacco) leaves, predominantly within the leaf mesophyll cells and without any significant alterations to their growth and seed setting properties. If translatable to high biomass crops, these initial levels would already equate to oil yields per hectare that exceed those of most existing oilseed crops. Such highly productive vegetative plant oil production platforms could provide a step-change in the bio-based energy and materials landscape, opening up prospects of achieving a sustainable, scalable and affordable raw material supply for industry without compromising on supply of food oils from conventional sources.

Engineering Increased Plant Diacylglycerol Acyltransferase Accumulation in Baker's Yeast. M.S. Greer (*Biotechnology Division Student Award Winner*) and R.J. Weselake, Agricultural Lipid Biotechnology Program, Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

Recently, there has been great interest in increasing triacylglycerol (TAG) accumulation in both micro-organisms and plants to provide edible oil and/or feedstock for production of biofuels and other bioproducts. Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of *sn*-1,2-diacylglycerol to produce TAG. Expression of modified *Brassica napus DGAT1* coding sequences in *Saccharomyces cerevisiae* revealed that the amino acid sequence in the N-terminal region of these enzymes can alter their *in vivo* accumulation, resulting in up to a two-fold increase in cellular TAG content. Placement of an N-terminal tag, containing poly-histidine and a second epitope, on the various forms of BnaDGAT1 stabilized accumulation of the enzymes, irrespective of the amino acid sequence of their native N-terminal sequence. Microsomal fractions from *S. cerevisiae*, producing the high-accumulating enzymes, exhibited DGAT specific activities which were substantially greater than typically reported for recombinant production of DGATs in yeast. DGAT activity was further assayed based on mass spectrometric quantification of TAG isolated by high temperature gas chromatography. DGAT-catalyzed TAG production using this method was strongly correlated (R^2=0.99) with enzyme-catalyzed TAG production based on the use of radiolabeled acyl-CoA.

Solubilization and Purification of Recombinant *Brassica napus* Diacylglycerol Acyltransferase 1. K.M. Caldo[1], M.S. Greer[1], G. Chen[1], M.J. Lemieux[2], and R.J. Weselake[1], [1]Alberta Innovates Phytola Centre, Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Dept. of Biochemistry, University of Alberta, Edmonton, AB, Canada.

Diacylglycerol acyltransferase 1 (DGAT1) has been used in lipid biotechnology to modify oil deposition in a variety of organisms. Although the first DGAT gene was identified more than 15 years ago and despite its importance in lipid biochemistry, DGAT1 has not been subjected to intensive structure-function studies due to difficulties in purifying the membrane-bound enzyme. Herein, we describe a method for purifying recombinant poly-histidine tagged *Brassica napus* DGAT1, produced in *Saccharomyces cerevisiae*. n-Dodecyl-ß-D-maltopyranoside (DDM) was used to solubilize the enzyme from yeast microsomes. Cobalt affinity chromatography was employed to highly purify solubilized *Bna*DGAT1, the identity of which was verified through in gel tryptic digestion coupled with LC-MS/MS sequencing. After tag removal, the enzyme was purified to apparent homogeneity in active form using FPLC-Superdex 200 13/30. Analysis of gel filtration fractions by SDS-PAGE suggested that different DGAT1 oligomers in detergent micelles were resolved during the gel filtration process. A major symmetrical peak with apparent molecular mass of 213 kDa, was used for preliminary crystallization trials. *Bna*DGAT1 was purified about 70-fold over the solubilized fraction and exhibited a specific activity of 11.11±0.32 nmoles TAG/min/mg protein.

Engineered Oil Seed Crops with Fish Oil DHA Levels. S.P. Singh and J.R. Petrie, CSIRO Plant Industry, Canberra, ACT, Australia.

Omega-3 long chain (=C20) polyunsaturated fatty acid (omega-3 LC-PUFA) have critical roles in human health and development with studies indicating that deficiencies in these fatty acids can increase the risk or severity of cardiovascular and inflammatory diseases in particular. In order to meet the increasing demand for these oils there is an urgent need for an alternative and sustainable source of EPA and DHA. This talk will discuss recent progress in the production of the omega-3 LC-PUFA DHA, in plant seeds. Groups have reported good progress in engineering the C20 EPA with seed fatty acid levels similar to that observed in bulk fish oil (18%) although undesirable omega-6 PUFA levels have also remained high. The conversion of EPA to the particularly important C22 DHA, however, has been problematic with many attempts resulting in the accumulation of EPA/DPA but only a few percent of DHA. I will describe the production of fish-oil like levels of the C22 fatty acid DHA in seed oils of two oilseed crop species, camelina and canola, with high omega-3/omega-6 ratios. Importantly, these results were achieved using a single multi-gene construct which potentially will allow for a simpler pathway for deregulation and breeding. We consider this to be a breakthrough in the development of sustainable alternative sources of DHA.

Potential Benefits of a Novel Dietary PUFA, dihomo-gamma-linolenic acid (DGLA) in Cardiovascular Disease. H. Gallagher[1], I.A. Guschina[1], I. Khozin-Goldberg[2], S. Boussiba[2], D. Ramji[1], and J.L. Harwood[1], [1]Cardiff University, Cardiff, Wales, UK, [2]Ben-Gurion University of the Negev, Sede-Boqer Campus, Israel.

Atherosclerosis, the underlying cause of myocardial infarction, stroke and peripheral vascular disease, is responsible for about 34% of all deaths in the Western world. Atherosclerosis is a chronic inflammatory disorder and measures to control inflammation offer promising avenues for treatment. DGLA, an n-6 PUFA, has been found to reduce atherosclerotic development in a mouse model of this disease though the mechanism remains ill-defined. With the availability of DGLA from a mutant of *Parietochloris incisa* (a green alga of potential commercial importance) the possibility occurred of investigating DGLA affects at the molecular level – with the development of new dietary treatments as an end point. We have used macrophages in a robust model for atherosclerosis and monitored the effect of DGLA in comparison to other fatty acids. Inflammatory, cytokine and signalling pathway responses were monitored. Our data go some way to explaining the effectiveness of DGLA and its promise as a dietary component to reduce cardiovascular disease.

Development of Enzyme Cascade Reactions for the Conversion of Fatty Acids into Valuable Oleochemicals. U.T. Bornscheuer[1] and J.B. Park[2], [1]Institute of Biochemistry, University of Greifswald, Greifswald, Germany, [2]Ewha Womans University, Seoul, South Korea.

In this lecture an enzyme cascade reaction will be presented. By the successive action of oleate hydratase, alcohol dehydrogenase, Baeyer-Villiger monooxygenase and esterase (all expressed recombinantly in E. coli for whole cell biotransformation) we can access dicarboxylic acids and omega-hydroxy carboxylic acids from unsaturated fatty acids. This system is highly flexible as the proper choice of enzymes in the cascade can be used to direct the formation of desired products to meet the needs of the oleochemical industry.

Characterization of a Trifunctional Fatty Acid Desaturase from Oleaginous Filamentous Fungus *Mortierella alpina* 1S-4 Using a Yeast Expression System. H. Kikukawa[1], E. Sakuradani[1], S. Kishino[1], S.B. Park[2], A. Ando[3,4], J. Shima[4], M. Ochiai[5], S. Shimizu[6], and J. Ogawa[1,4], [1]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan, [2]Laboratory of Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan, [3]Research Unit for Physiological Chemistry, Kyoto University, Sakyo-ku, Kyoto, Japan, [4]Research Division of Microbial Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan, [5]Suntory Global Innovation Center Ltd., Shimamoto-cho, Mishima-gun, Osaka, Japan, [6]Faculty of Bioenvironmental Science, Kyoto Gakuen University, Kameoka, Kyoto, Japan.

Mortierella alpina 1S-4 is capable of producing triacylglycerols rich in polyunsaturated fatty acids (PUFAs) including arachidonic acid (ARA). On the other hand, this fungus accumulates a small amount of eicosapentaenoic acid (EPA) with 0.3 g/L of culture broth and 10% in total fatty acids below a cultural temperature of 20°C. A ω3-fatty acid desaturase gene (*maw3*) which is involved in biosynthesis of n-3 PUFAs such as EPA was previously isolated from *M. alpina* 1S-4. In this research, we investigated the products of MAW3 catalyzing reaction in the yeast transformant. Two unknown fatty acids *de novo* synthesized in the yeast transformant expressing *maw3* gene were identified as n-4 hexadecadienoic acid (16:2^{9cis,12cis}) and n-1 hexadecatrienoic acid (16:3^{9cis,12cis,15}) by GC-MS and ^{1}H-NMR analyses. The MAW3 was demonstrated to be a trifunctional Δ12/Δ15/ω3-desaturase, exhibiting Δ12-desaturation for 16:1^{9cis}, Δ15-desaturation for 16- and 18-carbon fatty acids that had a preexisting *cis*-double bond at Δ12 position, and ω3-desaturation for 20-carbon fatty acids having that at Δ14-position.

Lipid Oxidation Can Cause Aberrant Results in Moisture & Oil Content Determination of Oilseeds Particularly High ω3 Sources. D. Hildebrand[1], S. Patel[1], M. Ma[1], M. Al-Amery[1], and P. Armstrong[2], [1]University of Kentucky, Lexington, KY, USA, [2]USDA-ARS, Peoria, IL, USA.

Seeds generally range from 10 – 15% moisture at harvest and are often dried further for storage. Very long term storage can be achieved at temperatures below freezing and = 7-8% moisture. This can be achieved by mild heating, freeze drying or with desiccants. To achieve complete removal of free water and determine the moisture content for calculation of components on a dry weight basis it is standard to heat seeds at 101 – 110°C until the weight stops changing. Oilseeds are mainly valued for the oil and protein components. In drying

oilseeds with high polyunsaturated fatty acid levels at 103°C we find oil contents can decrease with drying especially in seeds with high tri-unsaturated levels. Oil measured by NMR and NIR can nearly disappear and this is correlated with loss of the di- and tri-unsaturated fatty acids linoleate and linolenate. During drying we find seed weights can actually increase temporarily and then decrease apparently due to oxygen adsorption and then both moisture and volatile lipid oxidation product loss. Thus drying by standard methods not only can render oil content determinations inaccurate but also make the moisture content measurement imprecise. This problem is greater with ground than whole seeds. This problem can be avoided by drying in an atmosphere of nitrogen (N2) gas.

BIO 5: General Biotechnology

Chairs: D.G. Hayes, University of Tennessee, USA; K. Takahashi, Hokkaido University, Japan; and L. Kleiner, Bunge North America, Inc., USA

Lipid Composition of the Liposome Membrane Largely Affects the Transport and Absorption of the Liposomes Through Small Intestinal Epithelial Cell Model. Y. Konishi and K. Takahashi*, Hokkaido University, Hakodate, Hokkaido, Japan.

Mechanisms involved in transportation and uptake of several kinds of omega 3 phospholipid liposomes through small intestinal epithelial cell monolayer models (Caco-2 model and M cell induced model) were examined through using endocytosis inhibitors (EIPA and chlorpromazine). Liposomes were prepared using PC obtained from squid, the PS transphosphatidylated from that PC, and SQDG obtained from alga. The PC/PS and PC/SQDG liposomes, both the MLV and SUV show higher uptake not only in the M cell induced model, but also in the Caco-2 model. On the other hand, they showed only higher transport through M cell induced model. The transports of PC-MLV through both models were not inhibited by chlorpromazine, but on the contrary inhibited by EIPA in the M cell induced model. Thus PC-MLV is considered to be transported through M cell induced model by macropinocytosis. The transports of PC/SQDG-MLV through both models were not inhibited by the inhibitors. PC/SQDG-MLV seemed to open tight junction. It was considered that PC/PS-MLV may be transported by phagocytosis because the inhibitors did not affect. The uptake of all the liposomes examined was not inhibited by both inhibitors. It is considered that PC-MLV, PC/PS-MLV, and PC/SQDG-MLV are up taken by phagocytosis.

Studies of Oleaginous Filamentous Fungus *Mortierella alpina* for Useful Polyunsaturated Fatty Acid Production. A. Ando[1], T. Okuda[2], E. Sakuradani[2], J. Shima[3], J. Ogawa[2], and S. Shimizu[2,4], [1]Research Unit for Physiological Chemistry, Kyoto University, Kyoto-shi, Kyoto-fu, Japan, [2]Division of Applied Life Sciences Graduate School of Agriculture, Kyoto University, Kyoto-shi, Kyoto-fu, Japan, [3]Research Division of Microbial Sciences, Kyoto University, Kyoto-shi, Kyoto-fu, Japan, [4]Dept. of Bioscience and Biotechnology, Faculty of Bioenvironmental Science, Kyoto Gakuen University, Kameoka-shi, Kyoto-fu, 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 systems for this fungus to improve the fatty acid composition. In this study, we demonstrate an useful polyunsaturated fatty acid production by oleaginous filamentous fungus *M. alpina* breeding.

A Novel Glycerophosphocholine Cholinephosphodiesterase and Glycerophosphoethanolamine Ethanolaminephosphodiesterase from *Streptomyces sanglieri*. D. Sugimori, K. Okuda, S. Mineta, and J. Ogasawara, Graduate School of Symbiotic Systems Science and Technology, Fukushima University, Fukushima, Japan.

Glycerophosphocholine cholinephosphodiesterase (GPC-CP) and glycerophosphoethanolamine ethanolaminephosphodiesterase (GPE-EP) hydrolyzes glycerophosphocholine (GPC) or glycerophosphoethanolamine (GPE) to glycerol and phosphocholine or phosphoethanolamine, respectively. We have discovered that *Streptomyces sanglieri* strain A14 produces a novel GPC-CP and GPE-EP extracellularly. Here we report characterization, gene cloning, and efficient production of these two enzymes. GPC-CP and GPE-EP were metal-ion independent and monomeric proteins (~70 kDa). GPC-CP preferred GPC and GPE as substrate; however, GPE-EP showed GPE-specific activity. The amino acid sequences of GPC-CP and GPE-EP showed 76% and 85% identity to those of phospholipases C (PLCs) from *Streptomyces* spp., respectively. However, we concluded that GPC-CP and GPE-EP are essentially different from PLC because of the difference in the substrate specificities. The amino acid sequence of GPC-CP showed 39% identity to that of GPE-EP. GPC-CP and GPE-EP were produced as an active protein by *Streptomyces lividans* or *E. coli* as a host cell, respectively.

The Novel Enzymes for Polyunsaturated Fatty Acid Saturation Involved in Conjugated Fatty Acid Production by Lactic Acid Bacteria. S. Kishino[1,2], M. Takeuchi[1], S.B. Park[2], A. Hirata[1], N. Kitamura[1], K. Yokozeki[2], S. Shimizu[1], and J. Ogawa[1], [1]Division of Applied Life Sciences Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Laboratory of Industrial Microbiology, Kyoto University, Kyoto, Japan.

Saturation metabolism of polyunsaturated fatty acids by gastrointestinal microbes is a detoxifying metabolism of anaerobic bacteria, such as lactic-acid bacteria. This saturation metabolism generates characteristic fatty acids, e.g., conjugated fatty acids and *trans*-fatty acids, which are well known to present in ruminant-derived foods.
Our analyses on conjugated fatty acid synthesis in representative gut bacteria, the lactic-acid bacteria, demonstrated that *Lactobacillus plantarum* AKU 1009a can transform the *cis*-9,*cis*-12 diene structure of C18 fatty acids into the conjugated diene structures *cis*-9,*trans*-11 and *trans*-9,*trans*-11. In addition, this strain can saturate these conjugated dienes into the *trans*-10 monoene. In cell-free extracts from this strain, we identified the enzymes involved in CLA synthesis. Three enzymes, CLA-HY, CLA-DH, and CLA-DC, are necessary for synthesis of conjugated fatty acids such as CLA. Through genomic analysis in *L. plantarum* WCFS1, we found that *cla-dh* and *cla-dc* are located in a cluster with another gene, *cla-er*. In light of this, we tried to identify the function of the gene product (CLA-ER) together with those of CLA-HY, CLA-DH, and CLA-DC and found that CLA-ER is the key enzyme for polyunsaturated fatty acid saturation.

A Novel Lysoplasmalogen-specific Phospholipase D. Y. Matsumoto[1], D. Sugimori[1], S. Sakasegawa[2], and H. Matsumoto[2], [1]Fukushima University, Fukushima-shi, Fukushima, Japan, [2]Asahi Kasei Pharma Corporation, Izunokuni-shi, Shizuoka, Japan.

Plasmalogen, a type of phospholipid, is a biomarker for dementia and cancer. At present, the amount of plasmalogen in a blood sample is determined by the HPLC method using ^{125}I; however, this method requires complicated processes and extensive analysis time. Here, we aim to develop a new enzymatic method for plasmalogen determination. We have previously reported that plasmalogen can be hydrolyzed to lysoplasmalogen by phospholipase A_1 (PLA_1) from *Streptomyces albidoflavus* NA297. A novel enzyme, lysoplasmalogen-specific phospholipase D (LPls-PLD), from an actinomycete, *Thermocrispum* sp. RD4668 was found. To identify the enzyme gene, genome sequencing of strain RD4668 was performed by next-generation sequencing and then a predicted ORF database was constructed. The LPls-PLD gene (*lpls-pld*) was identified from the ORF database using its peptide sequences analyzed by a Xevo QTOF MS system. The amino acid sequence of LPls-PLD shows 66% identity to that of glycerophosphodiester phosphodiesterase (UniProt accession no. D3Q1U5). LPls-PLD was extracellularly produced by *Streptomyces lividans* transformed using an expression vector, pUC702/*lpls-pld*. LPls-PLD showed no activity toward phosphatidylcholine, plasmalogen, sphingomyeline, and glycero-3-phosphocholine, so that the method using LPls-PLD and PLA_1 is useful for measuring plasmalogen in a blood sample.

Pancreatic Lipase Selectively Hydrolyses DPA over EPA and DHA Due to Location of Double Bond in the Fatty Acid Rather Than Regioselectivity. T.O. Akanbi *(Honored Student Award Winner)*, A.J. Sinclair, and C.J. Barrow, Deakin University, Victoria, Australia.

The enzymatic hydrolysis of canola, anchovy and seal oils with different types and amounts of polyunsaturated fatty acids were measured using porcine pancreatic lipase (PPL) to establish the fatty acid selectivity of PPL. Substrates were subjected to the same conditions of hydrolysis, with percent hydrolysis monitored using iatroscan and fatty acid selectivity monitored using gas chromatography (GC). Regardless of their distribution on the glycerol backbone, as monitored by 13C nuclear magnetic resonance (NMR), a-linolenic acid (ALA) and docosapentaenoic acid (DPA) were rapidly cleaved by PPL while eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and stearidonic acid (STA) were hydrolysed more slowly. Results show that PPL preferentially hydrolyses ALA and DPA over EPA, DHA and STA, and this selectivity is due to fatty acid rather than regioselectivity. The primary structural factor associated with resistance to PPL appears to be the distance of the first double bond from the ester linkage being hydrolysed. These results may be useful for using this enzyme to concentrate DPA from marine oils, separating it from EPA and DHA.

Modification of Stearidonic Acid Soybean Oil by Immobilized *Rhizomucor miehei* Lipase to Incorporate Caprylic Acid. E.A. Ifeduba (*Biotechnology Division Student Award Winner*) and

C.C. Akoh, University of Georgia, Athens, GA, USA.

Immobilized *sn*-1,3 specific *Rhizomucor miehei* lipase (RML) was used to catalyze the incorporation of caprylic acid (C8:0) into high stearidonic acid (SDA, C18:4n-3) soybean oil (SDASO) to form structured lipids (SLs). The effects of type of biocatalyst (Celite-, octyl-sepharose-, and Duolite-immobilized RML) and reaction temperature (30, 40, 50, and 60°C) on acidolysis and acyl migration were studied. Celite-immobilized RML (C-RML) at 50°C maximized C8:0 incorporation and minimized acyl migration compared to other treatments. The optimal substrate molar ratio (C8:0 to SDASO), incubation time, and enzyme load for SL synthesis by C-RML at 50°C was determined by response surface methodology to be 6:1, 24 h, and 20% weight of substrates, respectively. This optimum treatment was scaled-up in hexane or solvent-free reaction media using SDASO or an SDA-enriched concentrate as initial triacylglycerol substrates. This yielded various SLs with C8:0 contents ranging from 17.0 - 32.5 mol% and SDA contents ranging from 20.6 - 42.3 mol%. When digested, these SLs may deliver C8:0 for quick energy and SDA for heart health making them potentially valuable for medical and nutraceutical applications.

Enzymatic Esterification of Phenolic Acids. A. Schär, S. Gayathri, and L. Nyström, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland.

Phenolic acids are strong antioxidants, which have to be lipophilized through an esterification for the application in oil-based systems. Until now, with the exception of alkyl gallates, phenolipids are rarely applied in industrial scale. Hydroxycinnamic acid derivates with a hydroxyl group in para-position (incl. ferulic acid) are considered to be difficult to be esterified by lipases due to inhibitory effects. An efficient esterification of ferulic acid would therefore lead to a broader availability of ferulic acid esters and increase the chance of future investigation on safety, antioxidant potential in various systems, and finally industrial applications. We achieved an efficient esterification of ferulic acid with linear alcohols of a chain length from C2-C18 using a cheap commercial lipase in a non-polar system. Yields in the range of 79% to 92% within 72h of incubation were reached depending on the alcohol chain length. Further investigations with different phenolic acids were conducted to explore the influence of structural differences on the esterification using lipases. The position of the hydroxyl group, a saturation/unsaturation in the side chain, and the number of hydroxyl groups all have a significant influence on the esterification activity. Therefore, reaction conditions need to be optimized individually for each phenolic acid.

Optimized Synthesis of Structured Lipids by the Interesterification of Flaxseed Oil and Tricaprylin in Solvent-free Medium. M. Khodadadi and S. Kermasha, McGill University, Sainte-Anne-de-Bellevue, QC, Canada.

Lipase-catalyzed biosynthesis of structured lipids (SLs) in solvent-free medium (SFM) was carried out by the interesterification of flaxseed oil (FO) and tricaprylin (TC), using Lipozyme TL-IM from *Thermomyces lanuginosus*, as the biocatalyst. The bioconversion yield (%) of the medium-long-medium type SLs (MLM-SLs), including CLnC (C-caprylic and Ln-linolenic acids), CLaC (La-linoleic acid) and COC (O-oleic acid), considered as the desirable monitoring responses, was investigated. Multiple response surface methodology (MRSM) was used to obtain significant models for the responses, on the basis of a five level, five variable central composite rotatable design. Reaction parameters, including temperature (Tr, 45 to 65°C), TC to FO molar ratio (Mr, 2:1 to 6:1), enzyme concentration (Ec, 5 to 10%, w/w), reaction time (Rt, 4 to 28 h) and agitation speed (As, 150 to 350 rpm), were selected for the optimization of the process. Significant models for CLnC, CLaC and COC were determined after regression analysis, with backward elimination. After the verification of the predicted models, multiple-response surface optimization of the monitored variables, CLnC, CCLn, CLaC, CCLa, COC and CCO, has been carried out by means of desirability function and distance approaches.

BIO-P: Biotechnology Poster Session

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

1. **pH-dependency of Activity and Production of a Cold-active Lipase from *Pichia lynferdii* Y-7723.** J.H. Bae[1], M.H. Kwon[1], J.Y. Kim[1], C.T. Hou[2], and H.R. Kim[1], [1]School of Food Science and Biotechnology, Kyungpook National University, Daegu, Korea, [2]Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL, USA.

Lipases with abnormal functionalities such as high thermostability and optimal activity at extreme conditions gain special attentions because of their applicability in the restricted reaction conditions. In particular, cold-active lipase(CAL)s have gained special attentions in various industrial fields such as washer detergent, pharmaceutical catalyst, and production of structured lipid. However, production of CAL is mostly found from psychrophilic microorganisms. Recently we found a novel cold-active lipase from *Pichia lynferdii* Y-7723 which is mesophilic yeast strain (Production of a Novel Cold-Active Lipase from *Pichia lynferdii* Y-7723, Hak-Ryul Kim et al., J. Agric. Food Chem. 2010, 58, 1322–-1326). In this study, we investigated about pH-dependency of activity and production of a novel cold-active lipase from *P. lynferdii* Y-7723. Catalytic activity of lipase Y-7723 was highly dependent on pH and temperature. Production was also highly influenced by initial medium pH.

2. **Production of a Novel Antioxidant Furan Fatty Acid from 7,10-dihydroxy-8(E)-octadecenoic Acid.** C. Dasagrandhi[1](*Biotechnology Division Student Award Winneri),* H.M. Park[1], C.T. Hou[2], and H.R. Kim[1], [1]School of Food Science and Biotechnology, Kyungpook National University, Daegu, Korea, [2]Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL, USA.

Furan fatty acids (F-acids) gain attention since they are known to play important roles in biological systems including human. Specifically F-acids are known to have strong antioxidant activity like radical scavenging activity. Although widely distributed in mot biological systems, F-acids are trace components and their biosynthesis are complicated and quite difficult from sources. Based on biochemical study, they are considered to be an essential nutritional factor for mammals and should be provided through diet. Hence several studies reported chemical synthesis of furan fatty acids using chemical catalysts. However, chemical synthesis required complicated multisteps. In this study we developed a simple one step synthesis of a novel furan fatty acid 7,10 epoxy-octadeca-7,9-dienoic acid (EODA) from a dihydroxy fatty acid 7,10-dihydroxy-8(E)-Octadecenoic acid (DOD) by heat treatment. Structure of EODA was confirmed by GC/MS, NMR, FTIR analysis and maximum production yield under the reaction conditions of at 90°C, 24 hours reached 80%. As expected, EODA showed antioxidant activity in terms of scavenging activity.

3. **Production of *trans*-10,*cis*-12 Conjugated Linoleic Acid-enriched Triacylglycerols via Two-step Lipase-catalyzed Esterifications.** I. Kang[1], I. H. Kim[2], H.D. Choi[3], and B.H. Kim*[1], [1]Chung-Ang University, Anseong, Gyeonggi-Do, Republic of Korea, [2]Korea University, Seoul, Republic of Korea, [3]Korea Food Research Institute, Seongnam, Gyeonggi-Do, Republic of Korea.

The aim of this study was to synthesize *trans*-10,*cis*-12 conjugated linoleic acid (*t*10,*c*12-CLA)-enriched triacylglycerols (TAG) with potential anti-obesity effects via two-step enzymatic reactions. Commercial CLA mixtures, containing 33.3% *t*10,*c*12-CLA and 32.0% *cis*-9,*trans*-11 CLA (*c*9,*t*11-CLA) was esterifed with dodecan-1-ol for selectively removing the *c*9,*t*11 isomer in the form of dodecyl esters. The reaction was performed in a recirculating packed bed reactor, using an immobilized lipase from *Candida rugosa* as a biocatalyst. A free fatty acid fraction containing 54.7% *t*10,*c*12-CLA was produced under the best conditions (temperature, 20°C; substrate molar ratio (CLA mixtures to dodecan-1-ol), 1:1; water content, 0%; reaction time, 36 h) established in this study. The free fatty acid fraction enriched in *t*10,*c*12-CLA was esterified with glycerol for preparing *t*10,*c*12-CLA-enriched TAG. The reaction was performed in a vacuum stirred batch reactor, using an immobilized lipase from *C. antarctica* as a biocatalyst. The best combination of temperature, enzyme loading, and vacuum level was 60°C, 10% of the total weight of the substrates, and 0.4 kPa, respectively. The *t*10,*c*12-CLA-enriched TAG of ~95% was successfully synthesized in 12 h.

5. **Enrichment of Docosahexaenoic Acid from Tuna Oil via Lipase-mediated Esterification Under Pressurized Carbon Dioxide.** N. Ma[1,2], S.I. Hong[1,2], T.T. Zhao[1,2], D. S. No[1,2], C.T. Kim[3], and I.H. Kim[1,2], [1]Dept. of Food & Nutrition, Korea University, Seoul, Republic of Korea, [2]Dept. of Public Health Sciences, Seoul, Republic of Korea, [3]Korea Food Research Institute, Seongnam, Republic of Korea.

This study focused on the use of pressurized CO_2 as a reaction medium for the enrichment of docosahexaenoic acid (DHA) from tuna oil fatty acids via lipase-mediated esterification. The DHA concentration in tuna oil fatty acids used as a substrate was 20.9 wt%. Of the three lipases tested, Lipozyme RM IM from *R. miehei* was selected for further study. Enzyme loading, water addition, and reaction time were also explored. Near-supercritical CO_2, prepared at 25 °C and 8.3 MPa, was the most effective reagent tested for enriching DHA from the residual fatty acid fraction. In addition to near-supercritical CO_2, optimal conditions included addition of 0.2 wt% (based on total substrates) water, enzyme loading of 5 wt% (based on total substrates), and a reaction time of 18 h. The DHA concentration and recovery yield for the residual fatty acid fraction under these optimal conditions were 75.8 wt% and 81.0 wt%, respectively.

6. **Preparation of Phytosteryl Ester Containing Pinolenic Acid in a Solvent-free System Using Immobilized *Candida rugosa* lipase.** D.S. No[1,2], T.T. Zhao[1,2], E.J. Lee[1,2], and I.H. Kim[1,2], [1]Dept. of Food & Nutrition, Korea University, Seoul, Republic of Korea, [2]Dept. of Public Health Sciences, Graduate School, Korea University, Seoul, Republic of Korea.

Phytosteryl ester synthesized with pinolenic acid (PLA) from pine nut oil is expected to have features of both phytosterol and PLA. In this study, lipase from *Candida rugosa* (CRL) was immobilized and then used to optimize conditions for synthesis of phytosteryl ester containing PLA. Lewatit VP OC 1600, a macroporous hydrophobic resin, was selected as the best carrier, and the optimum condition for the immobilization of CRL was established. With immobilized CRL prepared, synthesis of phytosteryl ester with fatty acid from pine nut oil was carried out. Parameters investigated were temperature, molar ratio (phytosterol to fatty acid), enzyme loading, and vacuum. Optimum conditions for synthesis of phytosteryl ester were a temperature of 60°C, molar ratio of 1:4, enzyme loading of 10% (based on the total weight of the substrate), and pressure of 80 kPa. The maximum conversion of phytosteryl ester was ca. 93 mol % at the optimum condition.

7. **Inhibition of Lipases by γ-Oryzanol.** A. Schär, L. Schwarz, and L. Nyström, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland.

γ-Oryzanol is a mixture of various phytosterols esterified to a ferulic acid. This lipid soluble mixture of phenolics is found primarily in rice, and in addition to its health benefits (cholesterol lowering, anti-inflammatory activity) oryzanol is a good antioxidant in oil-based systems, including high temperature applications. The ferulic acid moiety of oryzanol is a hydroxycinnamic acid derivate that bears a hydroxyl group in para-position and a double bond in the side chain. This structural combination in some free phenolic acids has been demonstrated to inhibit lipases. Therefore, the aim of this study was to explore the possible effect of oryzanol on lipase activity. Inhibitory effect of oryzanol on for example a lipase from porcine pancreas was demonstrated in an O/W emulsion by monitoring the formation of free fatty acids in a pH-stat. However, the effect seems to be strongly dependent on the exposure time of the lipase to the oryzanol. As lipases in general are activated on the interface of water and lipids, amphiphilic molecules like oryzanol are in the vicinity of the substrate and enzyme to participate in the reaction. Therefore, oryzanol has a potential as lipase inhibitor, increasing stability of lipids during storage, and decreasing their gastrointestinal digestion.

8. **Enrichment of Refined Olive Oil with Palmitic Acid and Docosahexaenoic Acid to Produce Human Milk Fat Analogue.** R. Li and C.C. Akoh, University of Georgia, Athens, GA, USA.

Refined olive oil was enriched with palmitic acid (PA) and docosahaxaenoic acid (DHA) via lipase-catalyzed acidolysis reaction using Novozym 435 in hexane to increase its nutritive value. The incorporation reaction was optimized by response surface methodology. Three independent variables, reaction time (12, 18, and 24 h), temperature (50, 55, and 60°C), and substrate molar ratio (refined olive oil:DHA:PA = 1:1:6, 1:1:9, and 1:1:12) and three responses, total PA and DHA incorporation and PA content at *sn*-2 position were investigated. Results showed that PA was incorporated into the triacylglycerols (TAGs) of refined olive oil at up to 55.8% while incorporation of PA at *sn*-2 position and total DHA were found to be up to 33.6% and 3.5%,

respectively. Second-order models were generated for each of the three responses. A chi-square test verified that the predicted values from the models were not significantly different from the observed values using randomly selected conditions. The prediction power of the models was further confirmed by a solvent-free scale-up reaction. The produced structured lipids have potentials to be used in infant formula products.

9. **Production of New Food Flavor from Myrtenol and Myrtenal Using *Spodoptera litura* as Biocatalysts.** R. Motooka, A. Usami, and M. Miyazawa, Kinki University, Higashiosaka, Osaka, Japan.

In the course of our studies of generating bioactive compounds from natural products by biotransformation. We reported biotransformation of monoterpenoids by the larvae of common cutworm (*Spodoptera litura*).

(1*S*)-(+)- and (1*R*)-(-)-myrtenol (**1** and **2**), a [3.1.1] bicyclic monoterpene alcohol, are known to be an important and widespread terpenoids.

Therefore, in this study, we investigated biotransformation of compound **1** and **2** by the larvae common cutworm (*S.litura*). As a results, compound **1** was converted to (+)-myrtenal (**1-1**), (+)-myrtenic acid (**1-2**), (+)-(1*S*,5*S*,6*R*)-6-methyl-bicyclo[3.1.1]hept-2-ene-2,6-dimethanol (**1-3**) and (+)-(1*S*, *4S*, 5*R*)-2-pinene-4,10-diol (**1-4**). Similarly, compound **2** was converted to (-)-myrtenal (**2-1**), (-)-myrtenic acid (**2-2**) and (-)-(1*R*,5*R*,6*R*)-6-methyl-bicyclo[3.1.1]hept-2-ene-2,6-dimethanol (**2-3**).

In summary, *S.litura* were converted regio- and stereoselectively oxidation at C-10 position and hydroxylation at C-4, 9 position of (1*S*)-(+)- and (1*R*)-(-)-myrtenol.

10. **Plant Based Enzymatic Synthesis of Resolvin and Protectin Analogues.** T.R. Walsh, J.L. Adcock, and C.J. Barrow, Deakin University, Geelong, Victoria, Australia.

Resolvins and protectins have recently been discovered as potent mediators of inflammation. They have potential for use as treatment for chronic inflammatory diseases such as arthritis, cardiovascular disease and asthma. Biosynthesis of these molecules is believed to occur in mammals through biocatalytic oxidation of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), using enzymes such as lipoxygenase (LOX).

A large number of plants are also known to contain LOX enzymes; however, to date there has only been a limited number of plant derived enzymes that have been utilized to produce resolvins, protectins, or analogues of these molecules.

We have characterised the activity of a number of potentially useful LOX enzymes from readily available plant materials. The regiochemistry of the products formed from biocatalytic oxidation of EPA and DHA, which is a key factor of their biological activity, has been identified by a variety of analytical methods. These products have the potential for use as therapeutic agents against chronic inflammatory diseases, or as dietary supplements to help prevent such diseases.

11. **Conversion of Glycerol in Ethanol Stillage to 1,3-Propanediol.** K. Ratanapariyanuch[1], Y.Y. Shim[2], M. Haakensen[3], and M. Reaney[2], [1]Dept. of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Dept. of Plant Sciences, Saskatoon, SK, Canada, [3]Contango Strategies Ltd., Saskatoon, SK, Canada.

Stillage is a dilute aqueous mixture of organic and inorganic molecules including acetic acid, betaine, glycerol, glycerolphosphorylcholine, lactic acid, 1,3-propanediol and succinic acid produced as a product of ethanol fermentation. Bio-ethanol production facilities in the USA produce more than 350 billion liters of stillage annually and thus it could be a resource for the recovery of many useful compounds. It was observed that storing stillage at warm temperatures led to conversion of the glycerol and lactic acid to 1,3-propanediol and acetic acid, respectively. Interestingly, when *Lactobacillus panis* strains were isolated from the fermenting stillage solution, cultured and reintroduced to the stillage, improved conversion of glycerol to 1,3-propanediol was observed. It was discovered that modifying pH and oxygen levels, supplementation of additional glycerol and nutrients altered fermentation rate, efficiency, and conversion. The best conversion condition was under oxygen-depleted conditions at pH 5, with added glucose (0.1 M) and glycerol (1 M). Addition of vitamins (B2, B3, and B12) and freeze-dried stillage increased the total 1,3-propanediol yield. These demonstrated that the ethanol industry not only holds considerable promise as a source of commodity platform chemicals, but that the yields and forms of the compounds can be modified for further profit.

12. **Integration of Anaerobic/Aerobic Microbial**

System to Produce Lipids for Fuels. D.L. Fortela, R. Hernandez, M. Zappi, E. Revellame, W. Holmes, S. Dufreche, and R. Subramaniam, Dept. of Chemical Engineering, University of Louisiana, Lafayette, LA, USA.

Lipids from enhanced sludge have been shown as a promising feedstock in biodiesel production, but the relatively high cost of the carbon source substrates such as glucose and xylose in the lipid accumulation creates challenge impacting broad commercialization. In this study, the feasibility of utilizing as substrates the volatile fatty acids (VFAs) such as acetic acid, propionic acid and butyric acid produced from the anaerobic digestion of cellulose was examined. The envisioned integrated process is the production of lipids from lignocellulosic biomass while minimizing energy inputs to deconstruct the latter to yield assimilable carbon sources. The experiments were conducted in 5-L bioreactors. Experimental results show that the concentration of VFAs in the fermentation medium significantly affects the lipid content of the enhanced sludge from return activated sludge (RAS). Modeling of the lipid accumulation from VFAs and the anaerobic digestion of cellulose were then conducted to determine the kinetic equations used in the simulation of the integrated processes.

13. **Production of Non-animal Derived Vitamin D_3 by Using a Genetically Engineered Microalgae *Aurantiochytrium* sp. strain.** T. Ujihara and S. Mitsuhashi, Kyowa Hakko Bio Co., Ltd., Tsukuba, Ibaraki, Japan.

Vitamin D_3 is a fat-soluble vitamin involved in various important physiological functions in the human body such as calcium metabolism, and the demand in the nutritional field is growing. Vitamin D_3 is currently produced by chemical conversion from animal derived steroid compounds, however, due to the risk of viral contamination non-animal derived vitamin D_3 will be required. Although it is well-known that some fungi produce vitamin D_2 (ergosterol), vitamin D_3, a natural form of vitamin D in human body, is thought to be produced only in vertebrates.

In this study, we examined the production of vitamin D_3 from non-animal sources. Since vitamin D_3 is synthesized from 7-dehydrocholesterol (7DHC), a precursor of cholesterol, we screened cholesterol producers in various microorganisms and isolated a microalgae strain *Aurantiochytrium* sp. S442 which produced a significant amount of cholesterol. We identified a gene for 7DHC reductase in the strain and created a disruptant of the gene by homologous recombination. The disruptant grew as well as the parental strain and produced 7DHC instead of cholesterol. We isolated 7DHC from the cells and confirmed that 7DHC was easily converted to vitamin D_3 via UV radiation.

In conclusion, we constructed 7DHC producer strain and it would be a useful for the production of non-animal derived vitamin D_3.

14. **Synthesis of Sugar Ester Surfactant Analog and Evaluating Its Emulsion Properties.** K. Ren and B. Lamsal*, Iowa State University, Ames, IA, USA.

A Glucose-palmitic acid ester was synthesized with immobilized lipases from *Candida Antarctica* in 20% dimethyl sulfoxide and 80% 2-metyl-2-butanol solvent system at 55°C. The major product, suspected to be a surfactant-analog with a molecular weight of 464 g/mole was identified using thin layer chromatography and LC-MS. The crude product mixtureat at 0.5% w/w was tested for stability and droplet size distribution in the oil-in-water emulsion at 10:1 ratio of water to canola oil. This was compared with 0.5% lecithin in emulsion. Emulsion ability was taken as spectrophotometer absorbance of 100X diluted emulsion at 500 nm at time 0, and emulsion stability as relative slope of decrease in absorbance values for next 2 h. The emulsion activity of surfactant-analog product mixture was much lower at 0.25 absorbance value, compared to 0.91 of lecithin. However, the emulsion was more stable with sugar-ester analog with a destabilization slope of -0.0005, compared to lecithin at -0.002. Melvern particle size measurement of emulsion showed that emulsion stabilized with surfactant analog had peak droplet size of 40micron compared to 26 for lecithin system. We suspect purity of analog to be much smaller to be comparable with lecithin on equal weight basis. Optimization of esterification reaction, and purification of analog product will be further carried out.

15. **An Efficient Bioprocess for Phytosterol Extraction and Its Ester Derivatives Production by Using Domestic Agricultural Produce as Substrate**. S.W. Chang, Dept. of Medicinal Botanicals and Health Applications, Dayeh University, Dacun, Changhu, Taiwan.

A natural compound contained in various cereals and seeds accompany with cholesterol-lowering and anti-cancer function was called phytosterol. However, it cannot be synthesized by ourself; instead of we should uptake it from normal

diet. Since the lower solubility in oil-soluble foods is the major problem for further industrial application, a modification process on phytosterol to produce oil-soluble ester derivatives was therefore developed.

Plant sterol esters are well-known functional ingredients and have been reported to have beneficial effects on plasma cholesterol level for cosmetic, nutraceutical, and pharmaceutical applications. The present work, we planned to combine the traditional oil extraction and esterification to produce a multiple function edible oil product using domestic agricultural produce as substrate. The lipase AY was selected as an optimal biocatalyst with higher competitive activity toward campesterol in acetone. All materials were extracted at 27°C for 3 h and the highest conversion yield of ß-sitosteryl ester (81.6 ± 1.3%), campesteryl ester (82.7 ± 1.2%) and stigmasteryl ester (65.8 ± 8.3%) was obtained at 27°C, 0.73 g enzyme amount for 24 h using sesame as substrate.

16. **Omics: Evidence of Nitrogen Assimilation, Lipid Biosynthesis, and Energy Management for Higher Oil Palm Yield.** T.S. Huat[1], T.H. Fang[1], T.O.E. Keong[1], W.Y. Ching[1], N.B. Keat[1], T.N.L. Mei[1], C.F. Tim[2], H. Kulaveerasingam[1], and D.R. Appleton[1], [1]Sime Darby Technology Centre, Serdang, Selangor, Malaysia, [2]National University of Singapore, Singapore.

To better understand lipid biosynthesis in oil palm mesocarp, in particular the differences in gene regulation leading to and including de novo fatty acid biosynthesis, a multi-platform 'omics' technology was used to profile mesocarp transcripts, proteins and metabolites during six critical stages of fruit development in comparatively high-yielding (HY) and low-yielding (LY) oil palm populations. Amino acids were found to be significantly higher in HY palms during fruit maturation in concordance with up-regulation of several amino acid metabolism genes. Nucleotide precursor availability was also observed to be higher in HY at weeks 18-22 compared to LY fruits. 127 genes of interest (GOI) were identified to be key contributors to oil production in mesocarp with 32% involved in glycolysis, fatty acid biosynthesis and lipid assembly. Levels of metabolites involved in glycolysis revealed interesting divergence of flux towards glycerol-3-phosphate, while carbon utilization differences in the TCA cycle were proven by an increase in malic acid/citric acid ratio. Several proteins in oxidation phosphorylation and ATP biosynthesis were up-regulated in HY fruits. Apart from insights into the regulation of enhanced lipid production in oil palm, these results provide potentially useful metabolite yield markers and genes of interest to assist with genetic marker development for use in breeding programmes.