2009 Annual Meeting Abstracts

MONDAY

MORNING
BIO 1: Biocatalysis I
Chair(s): C.T. Hou, USDA, ARS, NCAUR, USA; and R.N. Patel, SLRP Associates, USA

Synthesis of Chiral Compounds by Biocatalysis. R.N. Patel[1,2], [1]SLRP Associates, USA, [2]Unimark Remedies, Mumbai, India

Chirality is a key factor in the efficacy of many drug products, and thus the production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceutical industry. Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. The advantages of biocatalysis over chemical synthesis are that enzyme-catalyzed reactions are often highly enantioselective and regioselective. They can be carried out at ambient temperature and atmospheric pressure, thus avoiding the use of more extreme conditions which could cause problems with isomerization, racemization, epimerization, and rearrangement. Microbial cells and enzymes derived there from can be immobilized and reused for many cycles. In addition, enzymes can be overexpressed to make biocatalyst processes economically efficient, and enzymes with modified activity can be tailor-made. The preparation of thermostable and pH stable enzymes by random and site-directed mutagenesis has lead to the production of novel biocatalysts. This presentation describe the preparation of chiral intermediates for the synthesis various pharmaceutical.

Effect of Surfactants on Production of Oxygenated Unsaturated Fatty Acids by *Bacillus megaterium* ALA2. B.S. Kim[1], H.R. Kim[2], K. Ray[3], C.T. Hou[3], [1]Chungbuk National University, Chungbuk, Korea, [2]Kyungpook National University, Daegu, Korea, [3]NCAUR, USDA, Peoria, IL. USA

Bacillus megaterium ALA2 produces many oxygenated unsaturated fatty acids from linoleic acid. Its major product, 12,13,17-trihydroxy-9(Z)-octadecenoic acid (12,13,17-THOA) inhibits the growth of some plant pathogenic fungi. Because hydrophobic fatty acids need to be evenly dispersed in culture for effective contact with microbial cells, we investigated the effect of surfactants on the production of 12,13,17-THOA. Surfactant type (SO-25, Tween-80 and Triton X-100) with concentrations from 0.01, to 1% were used to evaluate their effects on cell growth and production of 12,13,17-THOA. Among three surfactants tested, Triton X-100 decreased cell growth and 12,13,17-THOA production while Tween-80 at 0.5% increased near double of 12,13,17-THOA production over the control. When the concentration of substrate was changed with a fixed concentration of Tween-80 (0.5 %), maximum 12,13,17-THOA production was achieved at 1% of substrate. When different concentrations of Tween-80 were tested in a dissolved oxygen controlled fermentor, 12,13,17-THOA production reached maximum at 0.5% of surfactant with the productivity being 20% higher than the control and prevent further metabolism of product THOA.

Production of Lyso-phospolipids using Microbial Phospholipase. S.H. Yoon[1], M.K. Lee[2], J.S. Rhee[2], [1]Korea Food Research Institute, Seongma-Si, Korea, [2]Korea Advanced Institute of Science and Technology, Daejun-Si, Korea

An extracellular phospholipase A1 was isolated and purified from Serratia sp. The enzyme was shown to be a monomer (molecular mass of 43,000Da), and to have the highest lipolytic activity for phosphatidylserine among phosphoglycerides tested. Enzyme activity was completely inhibited by the addition of chelating agent such as EDTA, and the inhibited activity was fully recovered by addition of Ca++. The enzyme was stable up to 70°C whereas optimum reaction temperature was 50°C at pH 8.5. Phospholipids were hydrolyzed into lysophospholipids by phospholipase A1 in aqueous-solvent, two-phase, and emulsion systems. Reaction yield of lysophospholipid produced in two-phase system was higher than that in emulsion system. The highest catalytic activity and stability of the enzyme was shown in the reaction in butyl acetate among 13 organic solvents tested. In two-phase system, 20% phospholipid was completely hydrolyzed into lysophospholipid by phospholipase A1.

Bioconversion of Phospholipids in Supercritical Carbon Dioxide Media. Toru shina[1], kazuteru Onoyama[1], Yoshikazu Inoue[2], Koretaro Takahashi[1], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan, [2]Lipid Lab., Kawasaki, Japan

Phospholipase A1 from Aspergillus oryzae (SANKYO LIFETECH CO., LTD., Tokyo) mediated selective partial hydrolysis in supercritical carbon dioxide media using squid meal phosphatidylcholine (PC), a typical marine phospholipid rich in DHA exclusively in sn-2 position of the glycerol backbone as substrate, was practically viable to enrich DHA more. The principle of this reaction was cleaving off fatty acid moiety in sn-1 position leaving the DHA moiety in the sn-2 position of the glycerophospho-backbone. Another phospholipid bioconversion trial done in supercritical carbon dioxide media was the transphosphatidylation of squid meal (PC) to phosphatidylserine (PS) which is usually denoted as â??DHA-PSâ?, a typical brain health beneficial compound. This reaction was carried out by Phospholipase D from Streptomyces sp. (ASAHI KASEI CO., LTD., Shizuoka, Japan). Expectedly, the desired transphosphatidylation proceeded in the supercritical carbon dioxide media though both phospholipids are basically insoluble in the media. Bioconversion of lipids in supercritical carbon dioxide media must be one of the most highly potential alternative solvent free systems in the future.

Protein Engineering in Lipid Modification. Uwe T. Bornscheuer, Greifswald University, Greifswald, Germany

Lipases and related enzymes (esterase, different phospholipases) are currently used as biocatalysts in a broad range of lipid modifications [1]. In this lecture, the application of protein engineering methods will be shown for the improvement of lipases in the modification of fats and oils. One example deals with a lipase from Rhizopus oryzae (ROL), which was engineered to increase its stability toward lipid oxidation products such as aldehydes with the aim of improving its performance in oleochemical industries. Key to success was the saturation mutagenesis of selected Lys and His residues combined with a MTP-based high-throughput screening of stable variants [2]. In a second examples, the engineering of an esterase to create a lipase-like biocatalyst using a cassette-mutagenesis method will be covered [3]. In addition, the development and application of high-throughput screening methods will be highlighted.[1] Bornscheuer U.T. (Ed.) Enzymes in Lipid Modification, Wiley-VCH, Weinheim; Metzger, J.O., Bornscheuer, U.T. (2006), Appl. Microbiol. Biotechnol., 71, 13-22.[3] DiLorenzo, M., Hidalgo, A., Molina, R., Hermoso, J.A., Pirozzi, D., Bornscheuer, U.T. (2007), Appl. Environm. Microbiol. 73, 7291-7299.[3] Hidalgo, A., Schliessmann, A., Molina, R. Hermoso, J., Bornscheuer, U.T. (2008), Prot. Des. Eng. Sel., 21, 567-576.

Enzymatic Synthesis of a Hydroxyl Aliphatic Amino Acid, 4-Hydroxyisoleucine. J. Ogawa[1,2], M. Hibi[3], T. Kodera[4], S.V. Smirnov[5], K. Yokozeki[3,4], S. Shimizu[2], [1]Res. Div. Microb. Science, Kyoto University, Kyoto, Japan, [2]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [3]Lab. Ind. Microbiology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [4]Ajinomoto Co., Kawasaki, Japan, [5]Ajinomoto-Genetika Research Institute, Moscow, Russia

4-Hydroxyisoleucine (HIL) is a potential drug candidate for the treatment of diabetes and obesity. Enzymatic synthesis of HIL was investigated. Bacillus thuringiensis strain 2-e-2 was found to produce HIL from L-isoleucine (Ile) through direct hydroxylation. The enzyme catalyzing the hydroxylation was purified and characterized as a 2-ketoglutarate dependent dioxygenase. The Ile dioxygenase (IDO) gene was cloned and sequenced. Homologous genes were found in the genomes of B. thuringiensis, B. cereus, and B. weihenstephanensis. All the recombinant IDO were found to show hydroxylation activity of C4-position of Ile and produced a single HIL stereoisomer, 2S,3R,4S-HIL. Aldolase-transaminase coupling reaction was applied for HIL synthesis. Arthrobacter simplex AKU 626 was selected as a potential strain catalyzing aldol-condensation of acetaldehyde and 2-ketobutyrate. The aldol-condensation product was further transformed to HIL by transaminase with L-glutamate as an amino donor. The aldolase of A. simplex AKU 626 was purified and the gene encoding the aldolase was cloned and sequenced. The enzyme belonged to HpaI/HpcH-aldolase family.

Biotechnological Strategy for Nutritional Improvement of Cereal-based Materials with Polyunsaturated Fatty Acids and Pigments. M. Certik[1], Z. Adamechova[1], V. Hanusova[1], E. Breierova[2], [1]Faculty of Chemical and Food

Technology, Slovak University of Technology, Radlinskeho 9, 812 37 Bratislava, Slovak Republic, Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 845 38 Bratislava, Slovak Republic

Cereals represent a major food supply for humanity. Although these sources are rich in proteins and carbohydrates, many of them are deficient in several essential nutrients, such as polyunsaturated fatty acids (PUFAs) and carotenoid pigments. One possible approach how to enhance the content of PUFAs or carotenoids in cereal diet is based on biotechnological transformation of cereal materials by solid state fermentations. Selected filamentous Mucorales fungi were applied for conversion of numerous cereal substrates to bioproducts enriched with PUFAs. Thamnidium elegans was selected as a producer of gamma-linolenic acid (GLA) and Mortierella alpina as a producer of dihomo-gamma-linolenic acid (DGLA), arachidonic acid (AA) and eicosapentaenoic acid (EPA). On the other hand, a range of yeast species utilizing agroindustrial substrates were employed for formation of carotenoids, such as beta-carotene, torulene, torularhodine (Rhodotorula and Sporobolomyces strains) and astaxanthin (Phaffia species). Such naturally prepared cereal based bioproducts enriched with either PUFAs or carotenoid pigments may be used as an inexpensive food and feed supplement.The work was supported by grants VEGA No. 1/0747/08 and 2/7031/27 by grant AVPP-0043-07.

Preparation of Functional Phospholipids via Phospholipase D-catalyzed Transphosphatidylation. Masashi Hosokawa, Kounosuke Suzuri, Yukihiro Yamamoto, Kazuo Miyashita, Hokkaido University, Hakodate, Hokkaido, Japan

Phosphatidylglycerol (PG) is a naturally occurring phospholipid (PL) which is found primarily in plants and microorganisms. PG has excellent liposome-forming ability and is typically used as an emulsifier and material for drug delivery system. Because microorganisms and plants contain small amount of PG, it is difficult to isolate PG from natural sources on an industrial scale. Therefore, PG was synthesized from phosphatidylcholine (PC) via phospholipase D (PLD)-catalyzed transphosphatidylation. In especially, we attempted to synthesize PG in an aqueous system, because some organic solvents are unfavorable in industrial processes, especially in the food industry due to their toxicity and flammable properties. The yield of PG were 71 mol% and 68 mol% from soybean PC and egg yolk PC, respectively, under the optimum reaction conditions of 50 μmol PC, 10 mmol glycerol, 3 ml of acetate buffer, 1.6 U PLD, and 30 μmol $CaCl_2$ at 37°C for 48 h. In case of salmon roe PC with 14.3% eicosapentaenoic acid and 26.8% docosahexaenoic acid, the PG yield markedly increased 94 mol% by addition of 46 μmol α-tocopherol, although the PG yield was only 10% in absence of α-tocopherol.

Enzymatic Modification of Amaranth Oil for Possible Infant Formula Application. A.M. Pina-Rodriguez, C.C. Akoh, The University of Georgia, Athens, Georgia, USA

Amaranth oil is rich in linoleic, oleic, and palmitic acids. Structured lipids (SL) for specific applications can be prepared through chemical or enzymatic interesterification. A two step modification of Amaranth oil was explored to obtain a suitable breast milk fat analog. First, a Novozym® 435 (non-specific) enzymatic interesterification of ethyl palmitate and Amaranth oil significantly increased the total content of palmitic acid, reduced linoleic acid content, and increased the amount of palmitic acid at the sn-2 position of the SL product. Further, a specific enzymatic interesterification catalyzed by Lipozyme RM IM to incorporate desirable amount of DHA on the previously enriched Amaranth oil yielded a SL product that more closely resembled breast milk fat for possible infant formula application. The resulting milk fat analog was evaluated for physical and chemical properties. The aim of this study was to effectively modify Amaranth oil via lipase catalysis for use as an alternative fat source for infant formula and other infant products.

Production of Biologically Active 7, 10-Dihydroxy-8(E)-Octadecenoic Acid from Olive Oil by *Pseudomonas aeruginosa* PR3. Min-Jeong Suh[1], Jae-Han Bae[1], Bit-Na Kim[1], Ching T. Hou[2], Hak-Ryul Kim[1], [1]Dept of Animal Science and Biotechnology, Kyungpook National University, Daegu, Korea, [2]Microbial Genomic and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL USA

Hydroxy fatty acids have gained important attentions because of their special properties such as higher viscosity and reactivity compared with other non-hydroxy fatty acids. The new bacterial isolate Pseudomonas aeruginosa (PR3) had been reported to produce mono-, di-, and tri-hydroxy fatty acids from different fatty acids. Of those, 7,10-dihydroxy-

8(E)-octadecenoic acid (DOD) was produced with high yield from oleic acid by PR3. Up to now, the substrates used for microbial HFA production were free fatty acids. Recently, triacylglyceride, specifically triolein was efficiently utilized as a substrate for DOD production by PR3 indicating that vegetable oils containing oleic acid could be used as substrate for HFA production by PR3. In this study we used olive oil containing high content of oleic acid as a substrate for DOD production. Production yield of DOD under the optimized environmental conditions represented 50% over olive oil.

Unique Microbial Reactions for Conjugated Fatty Acid Production. Shigenobu Kishino[1,2], Si-Bum Park[1], Akinori Ando[2,3], Satoshi Sugimoto[2], Kousuke Mihara[2], Jun Ogawa[2,3], Kenzo Yokozeki[1], Sakayu Shimizu[2], [1]Laboratory Ind. Microbiolology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Division of Applied Life Sciences, Graduate School Agriculture, Kyoto University, Kyoto, Japan, [3]Res. Division Microb. Sciences, Kyoto University, Kyoto, Japan

Conjugated fatty acid has attracted many attentions as a novel type of biologically beneficial functional lipids. In nature, the contents of conjugated fatty acids are very low. We screened useful reactions for conjugated fatty acid production in microorganisms, and found several unique reactions.Lactic acid bacteria (Lactobacillus plantarum)L. plantarum converted octadecapolyenoic acids with cis-9,cis-12 double bonds to corresponding conjugated fatty acids with cis-9,trans-11 or trans-9,trans-11 double bonds via 10-hydroxy fatty acids as the intermediates. Linoleic acid (LA), α-linolenic acid (ALA), γ-linolenic acid (GLA), and stearidonic acid were well converted to corresponding conjugated fatty acids.Filamentous fungi (Delacroixia coronata)D. coronata produced conjugated linoleic acid (CLA) from trans-vaccenic acid (trans-11-18:1) through Δ9-desaturasion. The produced CLA was mainly cis-9,trans-11-CLA.Anaerobic bacteria (Clostridium bifermentans)C. bifermentans transformed polyunsaturated fatty acids (C18 and C20) with cis-ω6,cis-ω9 double bonds to two conjugated fatty acids with trans-ω7,cis-ω9 or trans-ω7,trans-ω9 double bonds. C. bifermentans transformed LA, ALA, GLA, stearidonic acid dihomo-γ-linolenic acid, arachidonic acid, and eicosapentaenoic acid to corresponding conjugated fatty acids.

AFTERNOON
BIO 2 / IOP 2.1: Biodiesel
Chair(s): H.C. Holm, Novozymes AS, Denmark; and B. Cooke, Dallas Group, USA

Continual Production of Biodiesel Fuel from Various Oils by Solvent-free Enzymatic System. Yomi Watanabe, Toshihiro Nagao, Yuji Shimada, Osaka Municipal Technical Research Institute, Osaka, Japan

The solvent-free BDF production system using immobilized *Candida antarctica* lipase has been developed. It has the following advantages; 1) high conversion (>95%) can be reached at 30 ^{o} C, 2) the amount of methanol is the smallest among other methods including chemical and supercritical liquid methods, 3) the production cost can be reduced to be competitive to the chemical methods by continuously using the immobilized lipase for over than 3 months, 4) it is free from waste water containing alkali or salts, 5) organic solvent free, and thus 6) pre and post processing can be minimized, 7) glycerol can easily be reused.By stepwise addition of methanol, vegetable oil (from soy bean, rapeseed, and corn), crude oil, used frying oil were converted to >95% FAMEs without any pretreatments. Palm oil was also converted to BDF continually at high degree of conversion. In order to convert materials containing large amount of FFA in addition to acylglycerols, such as acid oil, to FAME, two step conversion system is effective; the first step is the esterification of FFA to FAME and the second step is the transesterification of acylglycerols to FAME. These systems were successfully scaled up to 30 L, and thus were considered to be applicable for industrial production of BDF from various oils.

Present Situation of Biodiesel Research and Development in Brazil. L.P. Ramos, Department of Chemistry, Federal University of Paraná, Curitiba, PR, Brazil

Biodiesel research and development in Brazil has gone a long way ever since the National Program was launched in January 2005. The annual production rose from virtually nothing in the late 90â??s to nearly 500 million liters in

January 2008 as a result of a B2 mandate, which was raised to B3 in July 2008 and shall be further increased to B5 in January 2013, according to the current program timeline. These measures represent a biodiesel annual demand of 1.2 billion liters for B3 and more than 2 billion liters for B5 in 2013. To supply the biodiesel required for the B3 mandate, 51 plants of different sizes were built, which corresponded to an annual production capacity of 2.670 MTPY. Hence, it is clear that most of these plants were not in full operation and this was so because lipid sources were either too expensive or scarcely available at this time. Besides, 27 plants were under construction or being commissioned and another 25 plants were on project development stages, coming up to a potential biodiesel annual production capacity of 5.294 MTPY for 2008-2009. Based on these numbers, there are rumors that the Government intends to anticipate the B5 mandate to 2010 but this will depend on the current trends in the international oil market, as well as uncertainties in the raw material supply for the upcoming years.Support: FINEP, MCT, CNPq, FundaÃ§Ã£o AraucÃ¡ria

Enzymatic Production of Biodiesels: A Kinetic Study. Xuebing Xu, Aarhus University, Aarhus C, Denmark

Using enzyme for the biodiesel processing instead of chemical catalysts has raised high interest recently. There are a few heated concerns. One of them is the possibility to use non-edible oils, where they is unlikely easy for chemical catalysts. Using enzymes as catalysts, the reaction has to be conducted in low temperatures such as below 70 °C. Therefore, the reaction system can be complicated with multiple phases and glycerol inhibitions. In this talk, we will present the work focusing on kinetic study. The work will illustrate how the kinetics are related to the overall performance in model reaction systems.

Integrated Production for Biodiesel and PDO with Lipase-catalyzed Transesterification and Fermentation. Dehua Liu, Wei Du, Yan Sun, Department of Chemical Engineering, Tsinghua University, Beijing, China

Lipase-catalyzed transesterification from renewable oils for biodiesel production has some advantages over chemical-catalyzed approaches, such as environmental friendship, lower energy consumption. However, the low stability and the high cost of the lipase had been being regarded as the main hurdle to the industrialization of lipase-catalyzed biodiesel production. Tsinghua University has proposed a novel process, with which the operational life of the immobilized lipase could be improved over 50-fold than traditional enzymatic approaches. After the successful demonstration in a pilot plant with capacity of 200kg/d biodiesel, the first commercial facilities with capacity of 24,000ton/year was constructed in Hunan, China and it was put into operation on Dec. 8, 2006. More and more customers from Germany, Singapore, Korea, Thailand etc, are negotiating for technology license. As a by-product, glycerol will be yielded at about 10% of biodiesel during the process of biodiesel production. How to convert glycerol has become a common problem. It could be a promising way to produce 1,3-propanediol (PDO) from glycerol. PDO is a valuable chemical material and especially it could be copolymerizes with terephthalic acid (or methyl ester) to form polytrimethylene terephthalate (PTT). PTT has excellent properties compared to other polymers such as PET. Tsinghua University has proposed a novel flexible process for PDO production from glycerol or glucose, and the demonstration was finished in pilot plant at the end of 2003. A facility with capacity of 4,000 tons/year1,3-PDO is being run in Hunan Rivers Bioengineering company, China.

Environmental Sustainability Analysis of Biodiesel Production - A Comparative Analysis of Different Production Schemes. I.T. Herrmann, M. Hauschild, Technical University of Denmark, Lyngby, Denmark

Due to their generally positive carbon dioxide balance, biofuels are seen as one of the energy carriers in a more sustainable future transportation energy system, but how good is their environmental sustainability, and where lie the main potentials for improvement of their sustainability? Questions like these require a life cycle perspective on the biofuel - from the cradle (production of the agricultural feedstock) to the grave (use as fuel). An environmental life cycle assessment is performed on biodiesel to compare different production schemes including chemical and enzymatic esterification with the use of methanol or ethanol. The life cycle assessment includes all processes needed for the production, distribution and use of the biodiesel (the product system), and it includes all relevant environmental impacts from the product system, ranging from global impacts like climate change and loss of non-renewable resources over regional impacts like acidification, eutrophication and photochemical ozone to more local impacts like ecotoxicity and physical impacts like land use, to allow judging on the overall environmental sustainability of the biodiesel and to support identification of the main focus points for improvement of the environmental sustainability

Renewable Petroleum™ Products and Technologies: Production of UltraClean™ Diesel via Fermentation. Wei Huang, LS9, Inc., South San Francisco, CA, USA

The urgent need for renewable alternatives to petroleum has fueled global efforts to commercialize technologies for the conversion of abundant renewable biomass to liquid transportation fuels. In addition to chemical and thermochemical conversion approaches, biocatalytic conversion technologies are being aggressively developed. At LS9, we apply the basic principles of synthetic biology to engineer microbes to efficiently convert renewable carbohydrates directly to diesel and other petroleum derived products. The speaker will discuss the use fatty acid biosynthetic pathway as route to variety of products such as biodiesel. This talk will focus on fermentation and downstream process development and optimization, fuel performance, the underlying economics, GREET analysis, a contrast with alternative routes and technologies.

TUESDAY

AFTERNOON
BIO 3: Biocatalysis II
Chair(s): C.T. Hou, USDA, ARS, NCAUR, USA; and Y. Shimada, Osaka Municipal Technical Research Institute, Japan

Synthesis of Monoacylglycerols with Various Fatty Acids by Esterification with *Penicillium camembertii* Lipase under Reduced Pressure. Yomi Watanabe, Yuji Shimada, Osaka Municipal Technical Research Institute, Osaka, Japan

We have reported two reaction systems with *P. camembertii* lipase for synthesis of MAGs with unstable fatty acids (FAs). One is esterification at low temperature. The reaction synthesized MAG of conjugated linoleic acid (MAG-CLA) in a high yield, because a product MAG-CLA was excluded from the reaction system when the reaction was conducted at the temperature at which only MAG-CLA solidified. The other is esterification with dehydration (at 30^{o}C/5 mmHg). A decrease in the amount of water bound to the lipase by dehydration caused a change in its substrate specificity. A lipase with enough amounts of bound water recognized glycerol, FAs, and MAGs; thus, the lipase catalyzed synthesis of MAGs and conversion of MAGs to diacylglycerols (DAGs). Meanwhile, a lipase with a small amount of bound water recognized glycerol and FAs, but not MAGs; thus, the lipase catalyzed synthesis of MAGs, but not conversion of MAGs to DAGs. In this study, we observed that the esterification with *P. camembertii* lipase stopped when conducting at 40-50^{o}C/5 mmHg. This phenomenon was assumed to be owing to removal of essential bound water by strong dehydration. Controls of reaction temperature and reduced pressure enabled syntheses of MAGs with saturated and unsaturated FAs of C_{10} to C_{18} in a good yield at >95% esterification.

Lipase-Catalyzed Synthesis of Triacylglycerols Containing Hydroxy Fatty Acids. Thomas A. McKeon[1], Sung-Tae Kang[2], [1]USDA, ARS, WRRC, Albany, CA, USA, [2]Seoul National Technical University, Seoul, Korea

Acylglycerols in general are useful in drug delivery systems and as emulsifiers. Inclusion of hydroxy fatty acids in acylglycerols expand potential uses, as the hydroxy functionality imparts altered physical and chemical properties. We have previously described synthesis of mono-, and di-ricinoleoyl glycerols from castor oil, which is composed of 90% ricinoleate (12-hydroxy oleate), using controlled lipase digests of triricinoleoyl glycerol isolated from the oil. Acylglycerols containing 12-hydroxy stearate, derived from hydrogenation of ricinoleate, have uses including acyltransferase substrates, and emollients for cosmetics. Because 12-hydroxy stearate is not a major component of any oil, we have used lipase-catalyzed esterification of this fatty acid to glycerol to produce tri-12-hydroxystearoyl glycerol (THS), optimizing parameters for the esterification in a solvent free system. Lipozyme RMIM 60 from Rhizomucor miehei (RML), a 1,3-specific lipase, was used as the biocatalyst in this study, as it gave the highest yield of THS in preliminary experiments. The yield of THS was dependent on the molar ratio of free fatty acid (FFA) to glycerol (GL). At a molar ratio of 3:1 (FFA/GL), the enzyme-catalyzed reaction was highly efficient. Varying the ratio of 12-HSA to GL allows for the preferential synthesis of one of the glycerides. Water present in the enzyme

preparation seriously affected the lipase-catalyzed esterification. Enzyme reactions were examined at three different water activities (Aw), 0.11, 0.53, and 0.97. The highest rate for synthesizing THS was observed at Aw of 0.11 and the yield also increased with lower water activity. The yield of THS also depended on the temperature. Production of THS was roughly proportional to temperature up to 100°C and sharply decreased thereafter. Mono-12-hydroxystearin synthesis was nearly zero under optimum conditions. By carrying out the reaction under vacuum to remove water, there was an increase of about 10%, a yield of 75% of THS at 85°C.

Genetic Characterization and Modification for Improved Microbial Biosurfactants. D.K.Y. Solaiman, R.D. Ashby, J.A. Zerkowski, N.W. Gunther, ERRC, ARS, USDA, Wyndmoor, Pennsylvania, USA

The push for industrial adoption of biobased materials including biosurfactants has intensified in view of the increasing environmental demands and shifting geopolitical situation in oil-producing regions. Microbial glycolipids (i.e., sophorolipid (SL), rhamnolipid (RL) and mannosylerythritol lipid (MEL)) are attractive replacements for petroleum-based surfactants. Research efforts abound to develop cost-effective production system and to produce products of improved functional property to facilitate commercialization. In this presentation, we report a genetic engineering effort to improve the production of RL by a patented Pseudomonas chlororaphis strain that synthesizes RL only under static growth conditions. We introduced a bacterial hemoglobin gene (vgb) into the P. chlororaphis strain via electroporation in order to study the effects of increased intracellular oxygen (icO2) content on the RL synthesis. Preliminary results showed that the increased icO2 did not appreciably change the level of RL production. Study to determine the effect of the bacterial hemoglobin on RL composition is on-going. The results of this study should allow a better understanding of the regulation of RL biosynthesis to benefit efforts to optimize the production process or to manipulate the product composition.

Synthesis of Reversely Structured Lipids Possessing N-3 PUFA at Terminal Positions. C.D. Magnusson, G.G. Haraldsson, University of Iceland, Science Institute, Reykjavik, Iceland

The current work describes a chemoenzymatic synthesis of a novel type of structured acylglycerols possessing long-chain n-3 polyunsaturated fatty acids (PUFA) including pure EPA or DHA located at the terminal positions of the glycerol backbone. An immobilized Candida antarctica lipase was observed to acylate glycerol and 1-O-alkylglycerols exclusively to the primary alcoholic end positions with derivatives of pure EPA or DHA. A subsequent acylation with saturated fatty acids to the remaining mid position using a chemical coupling agent resulted in reversely structured LML (long-medium-long) type TAG or similarly structured AML (alkyl-medium-long) type ether lipids. Alternatively, structured TAG possessing pure EPA at the end positions and DHA at the mid position and, its analogue, with pure DHA at the end positions and EPA at the mid position, were prepared in excellent yields and regiopure by similar approach.

Enzymatic Concentration of Nutraceutical Seaweed Lipids. Ayumi Sho, Tokutake Sashima, Masashi Hosokawa, Kazuo Miyashita, Faculty of Fisheries Sciences, Hokkaido University, Japan

Seaweeds are one of the important living resources since ancient times and have been explored as sources of food, feed and medicine in the orient as well as the west. They being plants of unique structure and biochemical composition, seaweeds could be exploited for their multifunctional properties. Seaweeds have been thoroughly researched as sources of useful bioactive components like phycocolloids, proteins, vitamins and minerals. Recently, their lipids have drawn increased interest due to several health benefits they afford. The major lipid or lipid related compounds of seaweeds - include carotenoids like fucoxanthin and fatty acids including highly unsaturated omega-3 and omega-6 fatty acids - which have been reported to possess several physiologically important functions including anti-oxidative, anti-cancerous, anti-obesity and other properties. This presentation focuses mainly on composition and analysis of seaweed lipids along with special emphasis on their recovery and application. For the effective separation of seaweed lipids enzymatic process has been used. Alginase decomposed polysaccharide network of the brown seaweed to enable the effective extraction of the lipids. The extracted seaweed lipids showed the higher content of functional carotenoid, fucoxanthin, and other high value lipid compounds such as 18:4n-3, 20:5n-3, and 20:4n-6.

Synergy of Multiple Lipases and their Forms in Lipid Processing. Xuebing Xu, Aarhus University, Aarhus C, Denmark

Lipases have different properties such as different specificity towards fatty acids, regional positions, and glyceride moities. Lipases have different forms such as immobilized lipases and non-immobilized lipases. Besides lipases as protein can have synergistic effects in terms of protein stabiliztion and activity enhancement. The full story is far from understanding. In this work, we have conducted studies to apply dual lipase systems in lipid processing. We found that activities had been enhanced in terms of interesterification degrees. The reasons are still not fuly clear and probably different from case to case in terms of type of lipases, forms of lipases, and reaction systems. A few case studies will be provided to illustrate the possible reasons.

Modification of Lipid Composition by Genetic Engineering in Thraustochytrid. T. Aki, H. Iwasaka, H. Adachi, M. Nanko, H. Kawasaki, S. Kawamoto, T. Kakizono, K. Ono, Department of Molecular Biotechnology, ADSM, Hiroshima University, Higashi-Hiroshima, Japan

Thraustochytrid is a group of microheterotrophic eukaryotes found in the marine area. Its high growth ability and lipid productivity can be advantage of the industrial producer. We have isolated a number of thraustochytrid strains and identified most of those in genera *Thraustochytrium*, *Schizochytrium*, or *Aurantiochytrium* (recently separated from *Schizochytrium*). The latter two showed a high productivity of triglycerides rich in DHA and docosapentaenoic acid. Some isolates also accumulated astaxanthin and canthaxanthin in their cells. To improve the productivity and expand the further usage of thraustochytrids, genetic manipulation method has been developed. Integration of plasmid DNA carrying a bleomycin-resistant gene from actinomycetes into genomic DNA of *Aurantiochytrium* cells was successfully achieved by homologous recombination and yielded some transformants with antibiotics resistant phenotype. Disruption of PUFA biosynthetic gene in *Aurantiochytrium* cells was then tried, and resultant transformants showing auxotrophy to unsaturated fatty acids could be obtained. The introduction and disruption of lipid biosynthetic genes in thraustochytrids will thus enable us to improve lipid composition and fermentation efficiency through the elucidation of biosynthetic mechanism for the functional lipids.

WEDNESDAY

MORNING

BIO 4 / S&D 4.1: Biobased Surfactants

Chair(s): D. Hayes, University of Tennessee, USA; D. Solaiman, USDA, ARS, ERRC, USA; and G.A. Smith, Huntsman Performance Products, USA

Improved Poly(3-Hydroxybutyrate) Synthesis from Glycerol Substrates and the Effect of Sophorolipid Addition on Physical Properties. R.D. Ashby, D.K.Y. Solaiman, USDA, ARS, ERRC, Wyndmoor, PA USA

As biodiesel gains a larger market share, glycerol is produced in ever-increasing quantities as a co-product from chemical transesterification reactions. Our past work showed that by using glycerol as a fermentation feedstock, both bacterial storage polymers (*i.e.*, poly(hydroxyalkanoates), PHA) and microbial glycolipids (*i.e.*, sophorolipids, SLs) can be produced. Poly(3-hydroxybutyrate) (PHB) can be synthesized by a strain of *Pseudomonas oleovorans* from glycerol. Presently, we have attained maximum PHB yields of 1.3 g/L using 10-L fed-batch fermentations with a 2% initial glycerol concentration. This is a 117% increase compared to previous shake flask cultures. Sophorolipids possess surfactant properties and have several potential applications including in cleaning and cosmetic formulations. By adding SLs to solution cast PHB polymer films, SLs acted to reduce tensile strength, elongation and modulus of the polymer films by modifying film surface and porosity. SEM of the PHB:SL composite films revealed that SL addition caused a dimpled surface topography, and the size of the dimples was related to the concentration of SL added to the matrix. In addition, increased concentrations of SL increased film porosity thus providing potential for slow release applications.

Rhamnolipid Production by Denitrifying *Pseudomonas aeruginosa*. Lu-Kwang Ju, Neissa M. Pinzon, Maysam Sodagari, The University of Akron, Akron, OH, USA

Rhamnolipid biosurfactants have various industrial, environmental, and medical applications. Though commonly synthesized by Pseudomonas aeruginosa, rhamnolipids are expensive to produce due to the highly foaming nature and complex metabolic regulations involved. To employ high cell concentrations in the bioreactors while avoiding the excessive foaming associated with aeration, rhamnolipid production by denitrifying P. aeruginosa has been investigated. The studies so far have confirmed the feasibility of this approach but also identified several new challenges that are not encountered in common aerobic or anaerobic (fermentative) bioprocesses. While nitrate is highly water soluble for easy delivery to cells, online monitoring of the nitrate concentration in broth is not available. Methods need to be developed for enabling nitrate supplementation to match the varying cellular denitrification rate. In this presentation, we will summarize what we have learned, including the online NAD(P)H fluorescence profiles corresponding to nitrate consumption. With a newly isolated strain under the denitrifying condition, we also observed the excessive production of metabolites (not rhamnolipids or alginate) that made the broth extremely viscous. Characterization and, if successful, identification of the responsible metabolite(s) will also be presented.

Glycerin Oleate Ethoxylates: Physical Chemical and Performance Properties. George A. Smith, Prakasha Anantaneni, Patrick Weaver, Huntsman Performance Products, USA

There is a growing demand for cleaning products based on natural ingredients. The general public perception is natural ingredients pose less risk to human safety and better for the environment. There are also growing concerns about ingredients based on palm and coconut due to destruction of the rain forest and loss of biodiversity. Natural based surfactants derived from locally grown crops such as soybean, corn and canola could be of considerable interest in the North American detergent market.This paper will discuss the preparation of glycerin oleate ethoxylates (GOE) and their physiochemical and performance properties in detergent applications. Glycerin oleate can be prepared several different ways. Reacting fatty acids or methyl esters with glycerin using a base catalyst is probably the most common production scheme but leads to a complex mixture of mono, di and triglycerides. It is possible to prepare pure monoglycerides by reacting glycerin with acetone, followed by direct esterification and removal of the acetal protecting group but this process is expensive and difficult to scale to the large quantities required for the detergent market. We have found that reacting soybean oil with excess glycerin under base conditions followed by high temperature phase separation produces reasonably pure glycerin mono oleate (GMO) in good yields. GMO can not be ethoxylated using a conventional base catalyst. The catalyst hydrolyzes the ester bond to produce fatty acid and glycerin. To avoid the hydrolysis reaction, GMO can be ethoxylated using a calcium based catalyst. The catalyst adds ethylene oxide of the primary and secondary hydroxyl groups and inserts EO into the ester functionality. The surface properties of glycerin oleate ethoxylates (GOE) was compared to convention alcohol ethoxylates. At the same degree of ethoxylation, GOEs have a lower CMC, cloud point and foam potential than LAEs based on midcut alcohol due to the longer alkyl chain length. GOEs show surface behavior similar to LAEs. Surface and interfacial tension increase with increasing degree of ethoxylation. GOEs show similar detergency to LAEs in single surfactant and multi-component systems.

New Type of Vegetable Oil Ethoxylates for Detergent Application. Raymond W. Cen, Prakasha Anataneni, George A. Smith, Patrick L. Weaver, Huntsman Corporation, 8600 Gosling Rd, The Woodlands, TX 77381, USA

Our Quest for plant-based surfactants continue. We have synthesized a new type of vegetable oil ethoxylates (EVOEs) by reacting epoxidized vegetable oils with EO and/or PO through a ring opening mechanism. EVOEs were found retaining a triglyceride structure and exhibit good surfactant perperties. EVOEs are cost effective for detergent and other HI&I applications.This presentaition will cover surface properties of EVOEs and compare them with those from methyl ester ethoxylates (MEE) and others. Several applications in detergent and hard surface cleaning are also discussed.This new type of vegetable oil ethoxylates can be manufactured cost effectively. So far they exhibit MEE-like performance there a viable choice for HI&I industry.

Biobased Surfactants and Emulsifiers with Antimicrobial Properties. Hans J. Altenbach, Rachid Ihizane, Bernd Jakob, Karsten Lange, Sukhendu Nandi, Manfred P. Schneider, Bergische Universitaet Wuppertal, Wuppertal, Germany

Agricultural crops provide a considerable reservoir of useful and low cost raw materials like fats and oils, plant proteins and carbohydrates. By selective combination of their molecular constituents (e.g. fatty acids, glycerol, amino

acids, saccharides) a wide variety of surface active materials can be prepared, all of them - due to their molecular constitution - being potentially highly biodegradable.In an attempt to acylate citric acid for the production of oil soluble derivatives we recently discovered that hydroxy carboxylic acids such as citric acid, malic acid and tartaric acid can be converted in one step and quantitatively into the corresponding O-acylated anhydrides, excellent electrophiles for ring opening reactions with the above nucleophiles from renewable resources, such as glycerol, sugar alcohols, amino acids and various carbohydrates including glucosamine. Next to novel surface active products- including gemini surfactants- several of the thus resulting molecules show additional benefits such as antibacterial properties and are thus potentially useful as multifunctional emulsifiers in cosmetics and food technology.The lecture will describe recent developments regarding novel combination products based on the above hydroxy carboxylic acids.

CANCELED - Characterization and Application of Methyl Ester Sulfonate Powder. Masahiko Matsubara, Yutaka Abe, Hiroyuki Masui, Kensuke Itakura, Ryoji Yasue, Tsutomu Ishikawa, Lion Corporation, Edogawa-Ku, Tokyo, Japan

A surfactant, Methyl Ester Sulfonate(MES) derived from natural fats and oils, shows high detergency at low concentration in hard water, and also has excellent biodegradability. MES is expected to be alternate surfactant to linear alkylbenzene sulfonate(LAS), because MES is an environment-friendly feedstock of surfactant produced from carbon-neutral vegetable oil. Until now, MES mixed detergent is mainly formulated with paste or dilute solution of MES. But these types of MES are more difficult to handle than other surfactant in the points of delivering MES to factory and mixing procedure into formulation. On the other hand, a highly-concentrated MES powder is interested in as a delivery form which can be directly mixed to powder detergent by dry-blend. But there is no detailed report about characters of MES powder and features of MES blended detergent. MES powder in laundry powder detergent shows good dispersibility, solubility and detergency. In this presentation, details of characterization of MES powder will be described. In addition, the detergents produced from other MES forms are compared to that including new MES powder in its properties and detergency. Furthermore, various factors which affect solubility and detergency will be reported.

Characterization and Application of Methyl Ester Ethoxylate. Hiroaki Shindo, Ryo Hyodo, Megumi Sadaie, Takahiro Okamoto, Hiromitu Takaoka, Lion Corporation, Edogawaku, Tokyo, Japan

In our previous report, we showed that Methyl Ester Ethoxylate (MEE) had good surfactant properties such as foaming, solubilization and wettability.Moreover, MEE showed good skin compatibility for hemolytic activity and environmentally-friendly because of good biodegradability. In this report, we found that laundry detergent including MEE as main surfactant has good detergency for sebum.And MEE has the advantage in the foam control, and in the low amount of residual surfactant on the clothes.As a result of good rinsing ability, MEE based detergent was able to reduce total volume of water consumption during the washing process.We would like to report these experimental results and discuss the mechanism.

Solvent-free Enzymatic Synthesis of Saccharide-fatty Acid Ester Surfactants: Bioreactor Design and Role of Supersaturated Solutions. Y. Ran, S.H. Pyo, D.G. Hayes, University of Tennessee, Knoxville, TN USA

Saccharide-fatty acid esters, biodegradable, biocompatible and biobased surfactants and value added products, were synthesized under low-water and solvent-free conditions at 65C in stirredbatchmode and using several different bioreactor systems that employed immobilized Rhizomucormiehei lipase (Lipozyme® IM, Novozymes, Franklinton, NC, USA) at 65C. The environmentally friendly approach, which takes advantage of the enhanced miscibility of acyl donor and acceptorsubstrates in the presence of ester, yields 80-85% ester and strong selectivity toward monoesters, a technical-grade biobased surfactant product that does not require further downstream purification. This presentation will focus upon the development and performance of bioreactor systems for their synthesis. Bioreactor systems that contained a packed column containing saccharide crystals and silica gel for delivery of saccharide provided similar yields to batch-mode reactions, but 3-fold lower rates. Subsequent experiments demonstrated the stirred-tank batch-mode bioreactor systems produced several-fold higher apparent saccharide concentrations due to the formation of stable ~100 micron-sized suspensions of saccharide crystals in solvent-free media compared to the concentrations yielded by the desorption column in the bioreactor systems.

AFTERNOON
BIO 5: General Biotechnology
Chair(s): R.N. Patel, SLRP Associates, USA; and J. Ogawa, Kyoto University, Japan

Palmitoleate Biosynthesis and Accumulation. J. Thoguru, G. Ratliff, S. Cheepineeti, D. Hildebrand, University of Kentucky, Lexington, KY, USA

Seed oils rich in monounsaturated fatty acids (MUFA) are not only important in human nutrition and as renewable chemicals. Seed oils rich in MUFAs (e.g. olive oil) usually have oleate (18:1) as the dominant MUFA. The shorter chain monounsaturated fatty acid palmitoleate (16:1) is more desirable in healthy foods and is superior in lubricant applications due to lower temperature fluidity. Palmitoleate is only abundant in the seed of some tropical species particularly *Macadamia integrifolia* (30%) and *Macfadyena unguis-cati* (65%). We have placed the 16:0-ACP desaturase gene from *M. unguis-cati* under a constitutive promoter and transformed this construct into leaves by Agro-infiltration. Analysis of transgenic leaves revealed that the best expressers only accumulate 16:1 to 3.5% of total fatty acids. In *M. unguis-cati* the accumulation of 16:1 starts at a mid-maturation stage. Initial results of lipid class distribution indicate that most of the 16:1 accumulates in triacylglycerol followed by phosphatidylcholine and phosphatidylethanolamine. In Macadamia 16:1 accumulation starts at the 5th stage of the seed development, which is a mid-maturation stage as was seen with *M. unguis-cati*. The amount of 16:1 levels increased from mid-maturation seed stage to mature seed stage. We have also studied lipids extracted from the pericarp (husk) of 8 developmental stages.

Enhancing the Genetic Diversity of Chia (*Salvia hispanica*). W. Jamboonsri, T. Phillips, D. Hildebrand, University of Kentucky, Lexington, KY, USA

There is considerable interest in chia as a health food and animal feed. It is a very high source of omega-3 fatty acids. Chia is a short-day plant and unable to set seed before frost in temperate areas. The aim of this study was to produce a new variety of chia which is able to flower during longer days. Chemical and physical mutagens (ethyl methanesulfonate; EMS and gamma ray respectively) were employed in mutagenesis. Mutagenized M_1 plants were grown and induced to flower by natural short day lengths in a greenhouse. M_2 seeds were planted in the field in the summer of 2008. Early flowering plants were found 55 days after planting while non-mutagenized plants had not produced any flower buds. 0.012% of EMS-treated M_2 population and 0.021% of the gamma treated population flowered earlier than the controls. We have demonstrated that early-flowering chia can be successfully produced by using EMS and gamma radiation and with a large enough M_1 and M_2 population size. Early flowering chia genotypes from this research work has potential to have positive impacts in several areas for example attracting new growers, building new markets, and benefiting consumer health in general.

Scale-Up Process for a Fed-batch Microbial Encapsulation of Fish Oil. Diliara R. Iassonova, Earl G. Hammond, Samuel E. Beattie, Iowa State University, Ames, Iowa, USA

A concentrated liquid yeast product containing fish oil encapsulated in *Cryptococcus curvatus* was produced by six 100-L fed-batch fermentations. It was reported earlier that *Cryptococcus curvatus* was able to grow on industrial-grade glycerol that was a byproduct of biodiesel production. Industrial-grade glycerol and urea were the sole carbon and nitrogen sources during the three-feeding steps growth stage of fermentation while menhaden oil was used as a carbon source during the two-feeding steps lipid accumulation stage of fermentation. Yeast biomass production was 54.9 g/L and yeast fat content was 59.2% on a cell dry weight basis. Yeast oil was predominantly triglycerides with significant amount of eicosapentaenoic (20:5,13.4%) and docosohexenoic (22:6, 6.1%) acids those were accumulated from substrate menhaden oil. In the yeast, all long chain polyunsaturated omega-3 fatty acids were stored in intracellular lipid droplets rather than as membrane lipids. Phosphotydilcholine was the major *Cryptococcus curvatus* phospholipid. The fatty acid composition of phospholipid fractions was similar for the growth and lipid accumulation stages of fermentation. Oleic and linoleic were the predominant fatty acids in the phospholipids fraction.

Driving Fast in Reverse: Accelerating the Development of Soybean Varieties with Enhanced Oil Traits. K. Bilyeu, USDA, ARS, Columbia, MO, USA

Plant breeding has been practiced first likely unintentionally and then driven by ever more sophisticated rationale. Many advances in crops have been achieved through plant breeding, especially forward genetic selection to identify superior traits in plants that exhibited phenotypic variation. More complex has been the identification of the genes responsible for the desired traits, although some success has been achieved. The use of model plant systems as a resource to build a knowledge base about basic physiological, biochemical, and molecular processes has provided the modern plant breeder with a new set of tools to use to create superior crops. Reverse genetics is a technique in which variants in candidate genes are sought to identify individual plants with predictably altered phenotypes. A positive and important outcome of this technique is that along with the desired phenotype, a perfect molecular marker can be easily generated and used to rapidly introgress the trait into elite crop varieties. The objective of this research was to develop a reverse genetics population for soybean and screen it for altered fatty acid profile traits using candidate genes for an increased oleic acid phenotype. The results indicate the power of the reverse genetics technique to complement existing plant breeding strategies.

Lipase Catalyzed Synthesis of Structured Analog of Platelet Activating Factor From Alkylglycerols. Carlos F. Torres, Luis Vazquez, Francisco J. Señorans, Guillermo Reglero, Sección Departamental Ciencias de la Alimentación, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain

Lipase-catalyzed transesterification of batyl alcohol with ethyl butyrate has been investigated. It was observed a very rapid consumption of batyl alcohol at the different reaction conditions studied (more than 85% w/w in only 5 min.) To shift the equilibrium of the transesterification reaction towards the diacylated alkylglycerol, distillation under controlled vacuum was utilized to remove the ethanol produced during the course of the reaction. Considering that vacuum also plays an important role on the rate of distillation of ethyl butyrate a compromise among the rate of evaporation of ethanol and that of ethyl butyrate was attained. Finally the transesterification reaction was scaled-up to produce up to ca. 500 g. of 2, 3-dibutyroil-1-O-alkylglycerols in three consecutive cycles. In this case the immobilized lipase was confined in mesh baskets to improve the reutilization of the biocatalyst.A kinetics modeling describing both the rate of transesterification and the rate of inactivation of the immobilized lipase was developed. The operational stability of the immobilized lipase (according to kd value attained), provides a lipase half-life of ca. 1500 hours.The present procedure is intended to be used for the synthesis of homogeneous and structured alkylglycerols with biological activities.

A Study on the Use of Ionic Liquids in the Enzymatic Synthesis of Bioactive Flavonoids with Antioxidant Properties. B.-M. Lue, Z. Guo, X. Xu, The University of Aarhus, Aarhus, Denmark

Ionic liquids (ILs) have been gaining momentum as an environmentally friendly alternative to traditional solvents; and while they have immense potential as solvents of the future given their thermal stability, low vapour pressure and adjustable solubility properties, there are also many challenges to be addressed when establishing an efficient reaction system.Major issues in the set-up of an efficient system for flavonoid ester biosynthesis, including (1) prediction of promising ILs from the vast number of possible candidates (using COSMO-RS), (2) experimental validation of IL candidates, (3) investigations into rheology, mass transfer phenomena and optimization of reaction parameters, as well as (4) water interactions in the reaction system, have been addressed. Antioxidant studies were also carried out to evaluate the potential of the ester products.Overall, the anionic moiety of the IL was found to be crucial in obtaining high flavonoid solubility; moreover, balancing solubility with acceptable lipase activity was essential in this system. Promising ILs were also found to have a stabilizing effect on lipase activity, exhibiting increased temperature optima compared with organic media. Finally, investigations into rheology, mass transfer and water state resulted in better insight and understanding of IL reaction systems.

Site-Directed Mutagenesis Improves the Thermostability of a Recombinant *Picrophilus torridus* Trehalose Synthase and Efficiency for the Production of Trehalose from Sweet Potato Starch. Shu-Wei Chang[1], Hsin-Hung Chou[2], Guan-Chiun Lee[3], Yi-Shan Chen[2], Tzunuan Yeh[4], Jei-Fu Shaw[4], [1]Department of Bioindustry Technology, Dayeh University, Dacun, Changhua, 515, Taiwan, [2]Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, 11529, Taiwan, [3]Department of Life Science, National Taiwan Normal University, Taipei, 116, Taiwan, [4]Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, 402, Taiwan

Trehalose synthase (TSase) has the catalytic ability for the reversible conversion of maltose to trehalose by intramolecular transglucosylation. Recently, a new recombinant Picrophilus torridus TSase (PTTS) gene was identified and synthesized from a hyperacidophilic, thermophilic archaea by using overlap extension PCR and transformed into Escherichia coli for protein expression. For the site-directed mutagenesis, we found that the replacement of N503 site with proline can increase the thermostability and pH stability of the recombinant PTTS. This mutant, N503P-PTTS, resulted in a 3.4 - fold higher half-life time than the wild type at 65°C reaction temperature. The optimum pH and temperature were also changed from 6 to 5.5 and 45 to 50°C, respectively. In an agricultural process application, the trehalose yield of mutant N503P-PTTS was 1.3-fold higher than the wild type with sweet potato starch as substrate at 50 ºC for 4 h. This suggests that the proline site substitution technology developed in this study might be potentially useful for altering enzyme properties and catalytic efficiency for use in industrial applications.

Enzymatic Production of Biodiesel: Eco-friendly Solution from Ionic Liquids. Zheng Guo[1], B.L.A. Prabhavathi Devi[2], Xuebing Xu[1], [1]Department of Molecular Biology, University of Aarhus, Aarhus, Denmark, [2]Lipid Science & Technology Division, Indian Institute of Chemical Technology, Hyderabad, India

As a renewable alternative of fossil fuel, the use of biodiesel has grown considerably due to increasing environmental concern and unprecedented high price of petroleum oil. This work investigated the potential of a special class of Ammoeng series ionic liquids, representing acyclic ammonium salts that contain cations with oligoethyleneglycol units of different chain length, for biocatalytic production of biodiesel. Distinct advantages (compared with traditional solvents) have been observed: only small amount of ILs needed for an efficient reaction (40-60 wt% of substrate); automatically phase separation for product recovery; over 98.5% biodiesel obtained without any post-processing; and incredibly low intermediate products formed. A theoretical characterization of biphasic oil/IL system has been performed by in-silico prediction and simulation with COSMO-RS. The results show that amphiphilic property of ILs create efficient interaction of oil and methanol; while larger partition difference of end products (glycerol and biodiesel) between the two phases constitutes the driving power (chemical potential) to move reaction to completion.

Esterification Conversion Rates of Oleic Acid, Crude Waste Oil, and Pre-treated Waste Oil Using Solid Resins Subjected to Single and Multiple Cycles. Sipho C. Ndlela, Iowa State University, BECON-Iowa Energy Center, Ames, IA, USA

This study compares the effect Amberlyst 15, 36, and 70 resins as catalysts in the esterification of oils, instead of sulfuric acid. Initially, oleic acid was subjected to an esterification reaction in the presence these resins. At the end of the 3 hour reaction period, Amberlyst 15 gave the best performance by having only 35% of free fatty acids remaining. However, after 1 hour using sulfuric acid catalyst, only 5-10% of free fatty acid remained. After the first cycle, the lifetimes of the resins in the reactor were evaluated using several consecutive esterification cycles. The second and third cycles generally suggested that conversion of fatty acids proceeded similarly to the first cycle described above. Although the resins were successful in converting high purity oleic acid, they did not perform as well on crude waste oil, which was attributed to fouling. Supplemental results from the reaction of waste oil using methanol and sulfuric acid catalyst suggested that 5% of the free fatty acids remained after two esterification cycles. A method currently being explored is to subject the waste oil to a fat splitting process at high pressure. The oil will then be pre-treated by a phosphoric acid and citric acid mixture, centrifuged, water washed, and dried prior to esterification using solid resins.

Biotechnology Posters

Chair(s): L. Pham, University of Philippines, Philippines

Crystallization and Oxidative Stability of the Structured Lipids from Rice Bran Oil, Fully Hydrogenated Soybean Oil and Coconut Oil through Lipase-Catalyzed Reaction.
P. Adhikari, J.A. Shin, K.T. Lee, Chungnam National University, Department of Food Science and Technology, Republic of Korea

Structured lipids (SLs) was produced using fully hydrogenated soybean oil (FHSBO) and rice bran oil (RBO) at different molar ratios of substrates [1:1 (RBO:FHSBO), 1:2 and 1:3]. Lipozyme TL IM (10% of total substrates) was used as a biocatalyst. Coconut oil (CO, 40% weight of total substrate) was added into all reaction mixtures as a source of medium chain fatty acid (MCFA). In DSC results, physical blends showed higher solid fat content (SFC) than SLs at each temperature. SFC of the SLs [(RBO:FHSBO) 1:1, 1:2, and 1:3] at 40°C were 0.4, 12.9 and 26.0%, respectively. Whereas their physical blend have higher SFC at 40°C, representing 53.8, 58.3 and 61.0%, respectively. After interesterification, β crystal form was disappeared and only β′ crystal form was observed. In rancimat test, the oxidative stability of the SLs was significantly lower than that of physical blends. When the catechin (200, 400 and 800 ppm) was added into the SLs, the induction period was significantly increased to 21.4, 34.1, and 44.3 h, respectively. In this study, hard fat stocks with a potential functionality as the shortenings and margarines were produced, and their oxidative stability was improved.

Purification of Lipase from Viscera of Sardine by Dye-Affinity Chromatography.

J.A Noriega-Rodríguez[1,2], H.S. García[2], O. Angulo-Guerrero[2], L.A. Medina-Juárez[3], A. Tejeda[3], N. Gámez-Meza[3], [1]Departamento de Ciencias Químico Biológicas y Agropecuarias., H. Caborca, Sonora. México, [2]Unidad de Investigación y Desarrollo en Alimentos, Veracruz, Ver. México, [3]Departamento de Investigaciones Científicas y Tecnológicas, Hermosillo, Sonora. México

A lipase was purified from the crude extract of viscera of sardine (Sardinops sagax caeruleus) by fractional precipitation followed by concentration by utrafiltration (30 kDa), affinity chromatography on Dye Matrex Blue A, and desalting by utrafilltration (10 kDa). The final enzyme preparation showed a single band with an apparent molecular mass of approx. 139.94 kDa by sodium dodecyl sulfate-polyacrilamide gel electrophoresis. The purified lipase from the viscera of sardine (LVS) had an optimal pH 7.0, and was active within the temperature range 20-50ºC, exhibiting an optimal of 30ºC. The LVS preferentially hydrolyzed Menhaden oil than olive oil as substrate, whereas microbial lipases preferred olive oil. These results suggest that LVS could be suitable for the application in the oleochemical industry for the preparation of structured lipids containing long chain polyunsaturated fatty acids.

Characterization of a New Pseudomonas Promoter for Gene Expression.

D.K.Y. Solaiman[1], B.M. Swingle[2], [1]ERRC, ARS, USDA, Wyndmoor, Pennsylvania, USA, [2]Robert W. Holley Center for Aging & Health, ARS, USDA, Ithaca, New York, USA

Bacteria in the Pseudomonas genus include industrially important strains such as those capable of fermentatively producing biopolymers (i.e., polyhydroxyalkanoates, PHAs) and biosurfactants (i.e., rhamnolipids, RLs) using agricultural feedstocks. Genetic engineering of these strains can result in an increased product yield or a modified product with the desired structure and property. Promoter sequence that drives gene expression is important in the genetic engineering of organism. In this study, ten P. syringae chromosomal sequences previously shown to function in Escherichia coli were individually cloned in front of a green fluorescent protein marker gene (gfp) in a plasmid. The recombinant plasmids were transformed into PHA-producing pseudomonads, i.e., P. resinovorans, P. corrugata and P. chlororaphis. Fluorescence measurement of the transformants showed that a putative promoter (termed P2) is capable of driving the expression of gfp gene in these pseudomonads. A computer analysis of the P2 sequence confirmed the presence of promoter-associated sequence-elements (i.e., -10 and -35 regions and a transcription factor-binding site, rpoD17). This promoter should be useful in the genetic engineering of the PHA-producing pseudomonads to improve the economics of PHA production.

Microbial Synthesis of Methyl-branched Poly(hydroxyalkanoate) Polymers from Methyl-branched Fatty Acid Substrates.

R.D. Ashby, H. Ngo, D.K.Y. Solaiman, USDA, ARS, ERRC, Wyndmoor, PA USA

Pseudomonas resinovorans was grown on 13-methyltetradecanoic acid (13-MTDA) and a mixture of isostearic acid (IA) isomers to produce methyl-branched medium-chain-length (*mcl*-) PHA polymers. Shake flask experiments revealed polymer productivities (the percent of the cell mass that is polymer) of 31±1% (n=3) and 23±3% (n=3) when grown in 13-MTDA and IA, respectively. Monomer content was determined by GC/MS of the acid hydrolyzed,

silylated methyl esters. Results showed that the *mcl*-PHA polymer derived from 13-MTDA was composed of 3-hydroxy-5-methylhexanoic acid ($C_{7:0}$; 4 mol%), 3-hydroxy-7-methyloctanoic acid ($C_{9:0}$; 67 mol%), 3-hydroxy-9-methyldecanoic acid ($C_{11:0}$; 16 mol%), 3-hydroxy-11-methyldodecanoic acid ($C_{13:0}$; 9 mol%) and 3-hydroxy-13-methyltetradecanoic acid ($C_{15:0}$; 2 mol%). The *mcl*-polymers synthesized from the IA isomeric mixture were more complex, containing both even and odd chain-length monomers with varying distributions of methyl-branched derivatives. The PHA distributions among the C8, C10, C12 and C14 carbon chain-length monomers included 3 isomers of C8, 5 isomers of C10, 7 isomers of C12 and 9 isomers of C14 each containing one linear-chain derivative and n–6 methyl-branched derivatives where n equals the total number of carbon atoms in each monomer unit (C8-C14).

Development of Oxidative Stability Improved Blends for Biodiesel.

R. de Guzman, H. Tang, S. Salley, K.Y.S. Ng, Wayne State University, Detroit, MI, USA

Biodiesel is an alternative fuel composed of saturated and unsaturated fatty acids that is very prone to oxidation attack. Exposure to air, heat, light and metallic contaminants results to the onset of autoxidation that degrades the fuel properties such as the kinematic viscosity, total acid number and the induction period. This study examines the effectiveness of a blended quaternary inhibitor system using binary (1 and 2) formulation of primary antioxidants from combinations of BHA, PG, PY, and TBHQ; a metal chelator (3) and an oxygen quencher (4) to properly address realistic oxidation conditions. Results indicate that the most effective binary antioxidant formulations exhibit synergisms to better protect the biodiesel (plant and animal based) as compared to using individual antioxidants. The addition of the metal chelator and the oxygen quencher improved the overall performance of the inhibitor system when used in biodiesel. The developed inhibitor system will then be investigated further under indoor and outdoor storage conditions for their effectivity as a function of time. The culmination of this study will lead us to achieve a better means to protect biodiesel against oxidation, which will be effective in both plant- and animal-based biodiesel, while still maintaining the overall quality within the approved specifications under realistic storage conditions.

Oxidative Stability of Structured Lipid Synthesized by Lipase-Catalyzed Interesterification.

Bo-Mi Lee[1], Mi-Hwa Chae[1], Ji-Eun Oh[1], Kwang-Il Kwon[2], Jong-Wook Kim[2], Hye-Kyung Park[2], Jee-Young Kim[2], So-Hee Kim[2], Jong-Hun Choi[1], In-Hwan Kim[1], [1]Korea University, Seoul, 136-703, Republic of Korea, [2]Korea Food & Drug Administration (KFDA), Seoul, 122-704, Republic of Korea

Oxidative stability of physical blend of the starting materials and of structured lipids produced by lipase-catalyzed interesterifications was investigated. The structured lipids were synthesized with fully hydrogenated soybean oil and high oleic sunflower oil in a weight ratio of 70:30 with the catalysis of Lipozyme TL IM (a silica-granulated *T. lanuginose* lipase) at 70°C in a packed bed reactor (PBR). Four structured lipids with different conversion degree were produced. Oxidative stability studies were carried out at storage temperature of 70°C during 25 days. In order to assess oxidative stability of the structured lipids, the changes in the peroxide value, anisidine value, and conjugated diene value were examined. There were differences in the oxidative stability between structured lipids with different conversion degree.

Lipase-Catalyzed Interesterification of High Oleic Sunflower Oil with a Fully Hydrogenated Fat in a Packed Bed Reactor Using Stepwise Changes in Temperature.

Mi-Hwa Chae[1], Bo-Mi Lee[1], Ji-Eun Oh[1], Hye-Kyung Park[2], Jee-Young Kim[2], Kwang-Il Kwon[2], Jong-Wook Kim[2], So-Hee Kim[2], Jong-Hun Choi[1], In-Hwan Kim[1], [1]Korea University, Seoul 136-703, Republic of Korea, [2]Korea Food & Drug Administration (KFDA), Seoul, 122-704, Republic of Korea

Lipase-catalyzed interesterification of high oleic sunflower oil and fully hydrogenated soybean oil was studied in a packed-bed reactor using a commercial immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM) as a biocatalyst. The interesterification reactions were conducted using either a constant temperature (70°C) protocol or a stepwise procedure involving two different temperatures, namely, 60 and 70°C. The interesterified products were monitored by analysis of triacylglycerol profiles, melting points, and solid fat contents. Conversion degrees are calculated as the ratio of the depletion in the concentration of tristearin relative to its corresponding initial

concentration. There were no significant differences in the conversion degree, melting points, and solid fat contents between a reaction held at a constant temperature of 70°C for 60 min and stepwise variation of the temperature from 70°C for 9 min followed by 60°C for 51 min. These results should make it possible to employ a lower reaction temperature, thereby decreasing the possibility of thermal deactivation of the enzyme and producing a corresponding reduction in energy requirements for the process.

Some Biochemical Properties of Lipase from Almond.

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A lipase was partial purified from the almond (Amygdalus communis L.) seed by ammonium sulfate fractionation and, dialysis. Kinetics of the enzyme activity versus substrate concentration showed typical lipase behavior, with Km and Vmax values of 25 mM and 113.63 Î¼mol min-1 mg-1 for tributyrin as substrate. All triglycerides were efficiently hydrolyzed by the enzyme. Almond seed lipase (ASL) was stable in the pH range of 6-9.5, with an optimum pH of 8.5. The enzyme was stable between 20 and 90Â°C, beyond which it lost activity progresively, and exhibited an optimum temperature for the hydrolysis of soy bean oil at 65Â°C. Based on the temperature activity data, the activation energy for the hydrolysis of soy bean oil was calculated as -5473.6 cal/mol. Soy bean oil served as good substrate for the enzyme and hydrolytic activity was enhanced by Ca2+, Fe2+, Mn2+, Co2+, and Ba2+, but strongly inhibited by Mg2+, Cu2+ and Ni2+. The partial purified enzyme retained its activity for more than 6 months at -20Â°C, beyond which it lost activity progressively.

Horseradish Peroxidase Degrades Lipid Hydroperoxides and Suppresses Lipid Peroxidation of Polyunsaturated Fatty Acids in the Presence of Phenolic Antioxidants.

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Lipid peroxidation in food causes decomposition of food. For prevention of peroxidation in food, α-Tocopherol is one of the most popular natural antioxidants. However, it is difficult to decompose previously generated lipid hydroperoxides with antioxidants. In this study, we show that linoleic acid hydroperoxide (LAOOH) was effectively degraded by horseradish peroxidase (HRP) in the presence of quercetin. Several natural phenolic antioxidants, such as quercetin, capsaicin, and α-tocopherol, acted as good hydrogen donors in the peroxidase reaction that occurs during lipid hydroperoxide degradation. However, glutathione, which is a non-phenolic antioxidant that acts as a hydrogen donor for glutathione peroxidase, could not suppress lipid peroxidation in the presence of HRP. Lipid hydroperoxides generated from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also degraded with HRP in the presence of quercetin, and oxidative decomposition of DHA was suppressed by this reaction.

Enzymatic Interesterification of Anhydrous Butterfat with Flaxseed Oil and Palm Stearin to Produce Low-*trans* Spreadable Fat.

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Low-*trans* spreadable fat (LTSF) was synthesized with anhydrous butterfat (ABF), flaxseed oil (FSO), and palm stearin (PS) by lipase-catalyzed interesterification. The synthesis of LTSF was carried out at 60°C, 180 rpm for 24 h in a shaking water bath. Two enzymes (Lipozyme RM IM and Novozyme SP435) were used as biocatalysts. Triacylglycerols (TAG) of ECN 36 (LnLnLn) and ECN 38 (LLnLn) increased as the FSO content in the ABF/PS/FSO blend ratio increased. ECN 42-46 increased after 24 h interesterification. Seven substrate mixtures for the production of LTSF were prepared: 12:6:2, 10:6:4, 9:6:6, 8:6:6, 6:6:8, 6:6:9, and 4:6:10 (by weight) ratio of ABF/PS/FSO. The α-linolenic acid contents (mol%) of each LTSF were 5.6 (12:6:2, ABF/PS/FSO), 10.6 (10:6:4), 14.9 (9:6:6), 15.8 (8:6:6), 20.8 (6:6:8), 22.1 (6:6:9) and 26% (4:6:10). The *trans* fatty acids in the LTSFs was less than 2%. Melting points of each LTSF and ABF were 37 (9:6:6, ABF/PS/FSO), 36 (8:6:6), 35 (6:6:8), 34 (6:6:9), 32 (4:6:10), and 34°C (ABF). Desirable substrate ratios for suitable melting range of LTSF were 8:6:6, 6:6:8, 6:6:9 and 4:6:10.

Cyclomaltodextrin Glucanotransferase-Catalyzed Transglycosylation from Dextrin to Sugar Esters and Alkanol Maltosides.

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Sucrose monolauroyl esters were found to serve as substrates for cyclodextrin glucanotransferase (CGTase)-catalyzed transglucosidation reactions, affording new sucrose esters that have an additional 1-3 glucose residues on the pyranose ring of the sucrose moiety in the ester.Maltosides of butanol, octanol, and lauryl alcohol were also found for the first time to serve as substrates for CGTase, and glycosyl residue was transfered from dextrin to the substrate affording novel maltosides with 3-4 glucose units. This ability of CGTase might potentially contribute to tuning of hydrophilic/lipophilic balance of alkyl glycosides affording neutral non-ionic surfactants with desired properties. These two enzymatic processes might give a new opportunity for environmentally safe production of saccharide-based more water-soluble amphiles.

Bioactive Oils Program ? An Integrated Approach to the Development of Novel Canola and Flax Seed Oils.

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Research on the nutritional effects of fats and oils over recent years has led to movement away from the use of trans fats in foods and toward the increased use of certain nutraceutic polyunsaturated fatty acids (PUFA) in the diet. The Bioactive Oils Program (BOP) represents a multidisciplinary approach to develop novel canola and flax seed oils to achieve these goals. The team has a plant breeder working on increasing the saturated fatty acid content of canola, complemented by molecular biologists working to improve the selectivity of TAG-biosynthetic enzymes and using a gene-inactivation strategy that can be incorporated into breeding programs. A molecular approach is also being used to develop flax seed with modified PUFA content. In parallel to these activities, analytical chemists and processing researchers are focusing on the development of novel antioxidants and extraction/processing procedures amenable to processing of the newly developed oils. In addition, nutritionists are examining the efficacy these oils in feeding trials using synthetic mixtures which will closely mimic the types of oils generated through the above approaches. Finally, the BOP team includes researchers focused on environmental risk assessment new crops and social scientists addressing issues such as consumer acceptability and intellectual property barriers.

Production of Ginsenosides Esters through Lipase (Novozyme 435)-Catalyzed Synthesis.

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Candida antarctica lipase B (Novozyme 435)-catalyzed synthesis of ginsenoside Rb1 (GRb1) esters was studied. In the preliminary experiment, we have studied the effects of three acyl donors with different carbon chains and three solvents on the GRb1 conversion rate. It was found that different acyl donors affected not only the degree of conversion but also the regioselectivity. Among the three solvent systems, the reaction in *tert*-amyl alcohol showed the highest conversion rate, while the reaction in the mixture solvent of *t*-BuOH and pyridine (1:1) had very low conversion rate. To allow the GRb1 lipophilicity, we decided to turn our attention to further study the optimal condition of synthesis of GRb1 with vinyl decanoate with 10 carbon chain fatty acids in *tert*-amyl alcohol. Response surface methodology (RSM) was employed to optimize such synthesis condition. From the ridge analysis with maximum responses, the maximum GRb1 conversion was predicted to be 61.51% in combination of factors (40.2 h, 52.95°C, substrate mole ratio 275.57, and enzyme amount 39.81 mg/mL). Further, the adequacy of the predicted model was examined by additional independent experiment at the predicted maximum synthesis conditions.

Preparation of *trans*-Free Stick Margarine Stock from Pine Nut Oil and Palm Stearin by Lipase-Catalyzed

Interesterification.
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Trans-free stick margarine was produced with pine nut oil (Pn) and palm stearin (Ps) in the weight ratios of Pn:Ps 50:50, 40:60, 30:70, and 20:80 by enzymatic interesterification. *Trans* fatty acid was not detected in the products. Major fatty acids detected were stearic (29.89-43.66%), and oleic (30.50-30.84%) acids. Both tocopherol and phytosterol were detected in the interesterified products. Pn:Ps of 40:60 and 30:70 were largely produced in a 1-L batch type reactor. Physical properties of the scale-up products were evaluated with melting and crystal behavior, solid fat content (SFC), slip melting point (SMP), hardness, crystal morphology, and polymorphic form. Solid fat content at 25°C were 23.6% (PN:PS, 40:60) and 36.2% (PN:PS, 30:70). Hardness of the scale-up products were 1281.3 g (PN:PS, 40:60) and 3733.0 g (PN:PS, 30:70), respectively. In crystal morphology, produced margarine stocks showed smaller crystal clusters comparing to blends. Desirable β′ form crystals were mainly observed in the interesterified margarine stock. Thus, the zero-*trans* stick margarine with desirable physicochemical properties can be prepared .

Enzymatic Interesterification of Perilla Oil with Soybean Oil: Optimization by Response Surface Methodology.
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Enzymatic interesterification was performed using soybean oil (SO) and perilla oil (Pe) as a source to enrich α-linolenic acid. Reaction condition was optimized using central composite design with reaction time (6-24 hr, X_1), reaction temperature (50-65°C, X_2) and substrate molar ratio (0.3-3.0, X_3). The reaction was carried out in a stirred-batch type reactor using Lipozyme RM IM. When variables were 8.87 hr (X_1), 57.6°C (X_2), and 1:0.8 ratio (X_3), maximum content of α-linolenic acid (ω3/ω6 ratio 1:1) was incorporated into SL. The predictive model for ω3/ω6 ratio, R^2 and P-value were observed 0.89 and 0.0132, respectively, the lack-of-fit was no significant. Fatty acid compositions at sn-2 position in SL were palmitic (0.92%), stearic (34.48%), oleic (0.43%), linoleic (34.42%), and α-linolenic acids (29.76%). To separate newly synthesized SL-triglycerides (TG) species, reversed-phase HPLC equipped with evaporated light scatting detector (ELSD) was used. In equivalent carbon number (ECN) of TAG profile, ECN 36 (LnLnLn), 38 (LnLnL), 40 (LnLnO), and 42 (unknown) were newly produced in SL. These results indicated that the synthesized SL could be a good source of α-linolenic acid for usage as vegetable oil or salad oil.

Solid Fat Content and Crystallization Behavior of Hard Fat Stock through Lipase-Catalyzed Reaction from Rice Bran Oil, Palm Stearin and Coconut Oil.
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Interesterification of rice bran oil (RBO) and palm stearin (PS) was performed at 65°C for 24 h using different molar ratios of substrates [(RBO:PS) 1:1, 1:2 and 1:3] with Lipozyme TL IM (10%, by weight of total substrates) as a biocatalyst. Also, coconut oil (CO, 40% weight of total substrates) was added to all reaction mixtures as a source of medium chain fatty acid (MCFA). MCFA (13.7-15.0%) was incorporated onto the triacylglycerol (TAG) backbone of interesterified products along with palmitic (30.1-36.8%), oleic (27.5-29.3%) and linoleic acids (10.5-15.4%). *Trans* fatty acid was below the detection limit of our analysis condition. Tocopherol and phytosterol were present in all interesterified products, having 3.3-7.7 mg/100g (tocopherols) and 138.4-274.4 mg/100g (phytosterols), respectively. Solid fat content (SFC) of the interesterified products (Product A, B, and C) at 25°C were 15.5, 25.6 and 34.2%, respectively. Physical blend showed higher SFC than the interesterified products at each temperature. POP and PPP (β-tending polymorphs) decreased after interesterification. In the XRD analysis, the interesterified products contained higher level of β′ polymorphic form, a desirable property for margarine.

Immobilization of Lipase from *Rhizopus arrhizus* to Produce Polyglycerol Polyricinoleate.
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Polyglycerol polyricinoleate (PGPR) is commonly used as emulsifier in the food industry. Known methods for preparing this compound adversely affect the quality of the final product which presents problems of coloration and odours. As an alternative, our research group is developing the enzymatic synthesis of PGPR by the catalytic action of one or more lipases (E.C.3.1.1.3). The enzymatic procedure consists of two steps. First, the ricinoleic acid is polymerized by the action of *Candida rugosa* lipase to obtain the estolide. This process has been optimized by the authors. Then the estolide is esterified with polyglycerol to yield PGPR. This second step is also catalyzed by a lipase. Among 24 different lipases, lipase from *Rhizopus arrhizus* has been selected because of its high catalytic activity and moderate price.The next step was the enzyme immobilization, and the optimization of the immobilization procedure is presented in this communication. Enzymes are usually immobilized to increase their thermodynamic stability and for easy separation from the reaction system in order to be reused. The immobilization has been carried out by adsorption on an anionic exchange resin Lewatit Monoplus MP 64 and the optimization of the immobilization procedure has included the study of the influence of pH and enzyme concentration, the study of the kinetics of the adsorption process and the determination of the adsorption isotherm. The different immobilized derivatives obtained have been used as biocatalyst in the reaction of esterification of condensed ricinoleic acid with polyglycerol to yield polyglycerol polyricinoleate with good results.

Structured Lipid Food Applications in Fried Sweet Potato Chips and in an Energy Bar.
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A structured lipid (RBOSL) was synthesized from rice bran oil (RBO) and caprylic acid with Lipozyme RM IM as biocatalyst. Sweet potato chips (SPC) were fried separately in RBOSL and RBO. Energy bars (EB) were formulated with RBOSL and RBO and a triangle test was conducted. Willingness to purchase sensory analysis was conducted on SPC and EB. Fatty acid profile and γ-oryzanol content of RBOSL after frying was not significantly different from the fatty acid profile before frying. SPC oil uptake was significantly higher in RBO than RBOSL. FFA and p-anisidine value of RBOSL was significantly higher in the RBOSL than in RBO. The color of SPC after frying in RBOSL was lighter and less yellow than the SPC fried in RBO. Triangle test results for SPC indicated no significant difference in the SPC fried in RBO and RBOSL at P≤.05 but was significant at P≤.10. Triangle test results for the EB indicated a significant difference between RBO and RBOSL formulations (P≤.05 and P≤.10). Results for the willingness to purchase (five point scale) sensory analysis of SPC and EB showed that the most frequent response was probably would buy. KEYWORDS: caprylic acid, enzymatic modification, Lipozyme RM IM, rice bran oil, sensory analysis, structured lipid, triangle test, willingness to purchase

The Characteristics of Desaturation of *Trichoderma* sp. M076 and formation of 9,12,15-hexadecatrienoic Acid (16:3ω1) through Δ15 Desaturation of 9,12-hexadecadienoic Acid (16:2ω4).
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Trichoderma sp. AM076, isolated from a fresh water, accumulate 9,12-*cis*-hexadecaenoic acid (16:2ω4), when grown with palmitoleic acid (16:1ω7). We here-in investigated the characteristics of desaturation in *Trichoderma* sp. AM076. Although 6,9,12-octadecatrienoic acid (18:3ω6) was detected when *Trichoderma* sp. AM076 was cultivated in the presence of 6,9-octadecadienoic acid (18:2ω9), the desaturation products of 6,9,12-octadecatrienoic acid (18:3ω6) and 6-octadecenoic acid (18:1Δ6) were not detected. These results suggest that the double bonds at the Δ6 position of 18:3ω6 and 18:1Δ6 disturb their Δ15 and Δ9 desaturation, respectively. This fungus also introduced a double bond at the Δ15 position of 9,12-hexadecadienoic acid (16:2ω4), thereby yielding a novel C16 polyunsaturated fatty acid (PUFA) identified as 9,12,15-hexadecatrienoic acid (16:3ω1). The results suggest that in this strain, the reaction that catalyzes the conversion of linoleic acid to linolenic acid, similar to the conversion of 16:2ω4 to 16:3ω1, is not ω3 desaturation but Δ15 desaturation.

Enhanced Enzymatic Hydrolysis of Lignocellulosic Biomass Pretreated by 4-Methylmorpholine N-oxide.
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This work reports the significantly improved enzymatic hydrolysis of lignocellulosic biomass, i.e., pure cellulose, switchgrass and corn stover, after pretreatment by 4-Methylmorpholine N-oxide (NMMO). More than 90% of cellulase

activity was maintained at an NMMO concentration lower than 0.5%. The significant improvement in enzymatic hydrolysis was achieved for pretreated biomass with or without removal of NMMO. For switchgrass and corn stover, the pretreatment improved the yields of reducing sugars by more than 50% and those of glucose by more than 2-3 folds after 24 h hydrolysis. Increases in kinetics and yields of sugars were also observed for pure cellulose after NMMO pretreatment. The NMMO pretreatment reduced the crystallinity of pure cellulose and possibly extracted cellulose out of switchgrass and corn stover. The modification of the biomass microstructure and possibly weakened physicochemical interactions between major components of biomass may have improved the accessibility of cellulases and efficiency of enzymatic hydrolysis. The tolerance of enzymes to NMMO and other possible decomposition products during pretreatments may be advantageous for future systematic development of NMMO-based pretreatment technologies to reduce the costs of lignocellulosic biofuels.

In vitro and _in vivo_ Control of Plant Pathogenic Bacteria of _Xanthomonas sp._ by the Volatile Seed Oil of _Poncirus trifoliata_ Rafin.

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The *in vitro* and *in vivo* efficacy of the volatile seed oil of *Poncirus trifoliata* was determined against plant pathogenic bacteria of *Xanthomonas* sp. In the applied*in vitro* tests, volatile seed oil of *P. trifoliata* at varied concentrations (5, 10, and 15 µl/disc) displayed potential antibacterial effect against the employed plant pathogenic bacteria of Xanthomonas sp. such as Xanthomonas campestris pv. compestris KC94-17-XCC, Xanthomonas campestris pv. vesicatoria YK93-4-XCV, Xanthomonas oryzae pv. oryzae KX019-XCO and Xanthomonas sp SK12 as a diameter of zones of inhibition (13.1 to 22.1 mm) along with MIC values ranging from 62.5 to 125 µg/ml. Further, in vivo elaborative study was also carried out against all the tested plant pathogenic bacteria of Xanthomonas sp. on greenhouse-grown tomato plants (Lycopersicon lycopersicum Mill.). At 1000 µg/ml concentrations, applied onto the leaves of tomato plants, essential oil displayed 100% inhibitory effect against Xanthomonas oryzae pv. oryzae KX019-XCO and Xanthomonas sp SK12. The results of this study support the potential role of volatile seed oil of P. trifoliata as natural antimicrobial agents for bio-controlling serve bacterial diseases in plants caused by Xanthomonas sp.

Enzymatic Interesterification of Lard with Soybean Oil: Characterization of Fats.

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The main goal of this study was to evaluate the physical-chemical properties of blends of lard and soybean oil modified by enzymatic interesterification catalyzed by Lipozyme TL IM lipase from Thermomyces lanuginosa in a tubular glass bioreactor with external jacket to maintain constant temperature and a fixed bed for support of enzyme for continuing interesterification. The increased of free fatty acids after continuous enzymatic interesterification is due to hydrolysis caused by present water in enzyme. The hydrolysis helps to remove the water from the enzyme, since it is consumed in the reaction. Degree of hydrolysis is inherent in the enzymatic interesterification, since the mechanism of the reaction involves hydrolysis followed by re-esterification of fatty acids released. Enzymatic interesterification produced new triacylglycerols that changed chemical-physical properties of the studied fats. Solid fat content, consistency, crystallized area and softening point of lard were increased after interesterification and this was due to the increase of the SSS+SSU triacylglycerols groups. Addition of soybean oil to lard promoted a decrease in solid fat content, consistency and melting point.

Enzymatic Production of Feruloylated Aclglycerols from Olive Fatty Acid Distillates as Sunscreen Agents.

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This work investigated the possibility of producing a suncreen agent based on ferulate glycerol esters with the incorporation of olive fatty acid distillate, glycerol and ethyl ferulate by using enzymatic processes. The effects of lipase type, dosage,temperature, free fatty acid to ethyl ferulate molar ratio and total glycerol to acyl-donor ratio on production of ferulate glycerol ester was obtained. The immobilized Novoyme 435 lipase of Candida antarctica was found to be the best performing lipase and it was observed that the reaction parameters of the reaction of 5% (w/w) of Novozyme 435, reaction temperature of 70°C, with a total glycerol to fatty acid and ethyl ferulate molar ratio of 1:2:1,

in presence of 3% (w/w) molecular sieves had resulted in an optimal yeild of 64.2%. The resulted ferulate glycerol ester mixture was partially purified through microfiltration and neutralization processes and this increase the yield to 70.1%. When analyzed through in-vitro analysis for its SPF value, the partially purified ferulate glycerol esters mixture had a protection factor of SPF 10.4 in the UVB region and a moderate protection in the UVA region

Lipase-Catalyzed Acidolysis of Olive Oil with Capric Acid: Effect of Water Activity on Acyl Migration.

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Structured lipids (SL) were synthesized by acidolysis of olive oil and capric acid with an immobilized lipase (Lipozyme TL IM) from *Thermomyces lanuginose*. The acidolysis reaction was carried out by incubating olive oil and capric acid with 1:3 mole ratio at 50°C. The effect of water content on the incorporation was investigated. The water activity range tested in this study was between 0.11 and 0.80. The capric acid incorporation into olive oil increased as the water activity increased, but acyl migration degree also increased. The degree of acyl migration of modified olive oils with similar degree of incorporation was investigated. For the similar degree of incorporation of approximately 40 mole%, there were significant ($P < 0.05$) differences in the degree of acyl migration. The effects of water content on the production of newly formed acylglycerols were also investigated.

Sea Cucumber Sphingoid Bases Induce Apoptosis via Inhibition of Phospho-AKT in Human Hepatoma Cells (HepG2).

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In this study, we investigated the anti-proliferative and apoptosis-inducing effects of sphingoid bases prepared from sea cucumber using human hepatoma HepG2 cells. Sea cucumber sphingoid bases markedly reduced the cell viability of HepG2 cells. DNA fragmentation of apoptosis indicators was observed in a dose-dependent manner. The expression level of apoptosis inducer Bax protein was increased by sphingoid bases. The GADD45, which play an important role in apoptosis-inducing pathway, was remarkably up-regulated by sphingoid bases. Up-regulation of PPARγ mRNA was also observed during apoptosis induced by sphingoid bases. The expression level of phosphor-AKT was decreased with the effects of sphingoid bases. These results suggest that sphingoid bases may induce apoptosis in HepG2 cells through up-regulation of Bax, GADD45, and PPARγ via inhibition of phospho-AKT.

Immobilization of Lipase and Its Application as Catalyst in the Production of Biodiesel.

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The extraction, purification and characterization of a lipase fraction from fishery processing discards and the enhancement of its activity and stability by immobilization as well as its application as a catalyst for biodiesel production was investigated.Lipase was extracted from the viscera of grey mullet; *Mugil cephalus* by ammonium sulfate fractionation followed by simultaneous desalting and concentration by ultrafiltration and immobilized onto a macroporous matrix. The effect of pH, temperature, time and enzyme load on immobilization was investigated. The immobilized lipase was characterized in terms of temperature and pH as well as its optimum conditions for enzymatic activity. The stability and efficiency of the immobilized lipase as a catalyst for biodiesel production was compared with a commercial immobilized lipase. This study was conducted to present an alternate and cheaper source of lipase, with the aim of offsetting the high cost associated with the use of commercial enzymes available for biodiesel production.

Synthesis of Amphipilic Star Polymers by PEGylation and Polymerization of Poly Hydroxy Fatty Acids.

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Unimolecular polymeric micelles, or UPMs, have been formed biocatalytically in our laboratory in an environmentally-friendly manner for potential utilization as drug delivery vehicles. The UPMs consist of a polyhydric alcohol (pentaerythritol) core, esterified to oligo(ricinoleic acid), and exhibit excellent physical and transport-related properties desired for viscosity index modifier ingredients of biolubricants, namely, high viscosity index and low melting point temperature. To further improve the performance of UPMs for drug delivery and lubricants, several approaches have been employed to increase the density of the grafted oligo(hydroxyacyl) chains onto the UPM's core. The first approach is by the enzymatic synthesis of triglycerides that contain the polymerizable undecenoic acyl group at the middle position and oligo(ricinoleic acid) and the 1 and 3-positions. These molecules can then be joined together via free radical polymerization. The first step, the *Candida antarctica* lipase-catalyzed synthesis of triundecenoate via use of undecenoic acid vinyl ester, has occurred successfully, at a > 95% yield. The second step, the *Rhizomucor miehei* lipase-catalyzed interesterification of triundecenoin and ricinoleic acid, has occurred at approximately 40-50% conversion. A second approach, the *Rhizomucor miehei* lipase-catalyzed interesterification of undecenoic acyl groups with castor oil, is currently under investigation.

Marker-assisted Selection for the Development of Low Phytate Soybeans.
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Development of low phytate soybean improves soy protein by providing enhanced nutrition and metabolism for poultry and swine. Low phytate soybean will be favorable to the environment by reducing phosphorous loads to agricultural lands and surface waters. The objective of this project was to develop a superior quality, high yielding soybean cultivar with low seed phytate. We used molecular marker assisted selection (MAS) at each backcross stage to facilitate genome recovery. Simple sequence repeat (SSR) markers have enabled us to i) transfer two recessive alleles governing the low phytate trait and ii) identify which specific individual backcross plants had DNA of the greatest commonality with the genome of the recurrent parent. We utilized two SSR loci, Satt237 (linkage group N) and Satt561 (linkage group L), for dual marker assisted selection for gene transfer of the low phytate trait. Additional molecular markers dispersed across the genome proved to be effective for facilitating genome recovery of the high yielding soybean cultivar recurrent parent ('USG 5601T') each backcross generation. Chemical analyses confirmed that the low phytate trait was inherited in concert with the molecular markers in some but not all progeny lines. Selected lines will be field tested in 2009. Our long-term goal is to develop a low phytate version of USG 5601T soybean.

Comparative Proteomic and System Biological Approach to Study Cell Wall Regeneration in *Oryza sativa*.
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The cell wall is the main source of cellulose, the most abundant and useful biopolymer on the earth. Nowadays, there is a growing recognition that cellulosic ethanol is an alternative to corn-based ethanol because the latter is unlikely to provide a long-term solution. Optimizing plant biomass for biofuels processing efficiently requires a thorough understanding of plant cell wall structures and functions, further applying genetic manipulations to modify the quality and quantity of cell wall components. To study the biological responses involved when cell wall removal and clarify cell wall dynamic regulations for cell development, we used shotgun proteomic approach and label-free quantification method to investigate the protein differential expression from *Oryza Sativa*, revealing 223 up-regulated proteins and 150 down-regulated proteins. Moreover, protein interaction network studies show that several cellular processes have close connections in response to cell wall removal and regrowth, like energy metabolism, cell growth and division, protein synthesis and transport, responses to stresses and chromosome reorganization. This work reports the establishment of a protein interaction network map using comparative proteomic approach combined with system analyses, which has provided valued insight into plant cell wall.

Nano-epoxy: a Two-component Molecular Glue for Lipid Vesicles.
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Artificial particulate systems such as lipid vesicles are found in a variety of biomedical applications such as drug delivery and targeting. More versatile layers of control would be added if liposomes could be glued together on demand while stabilized against fusion. Here we present a two-components molecular glue composed of a protein and calcium ions, each component specialized for fast and specific binding to negatively charged lipid membranes. Upon mixing the two components, the high affinity binding of this glue starts to tightly bridge two lipid vesicles on a sub-second scale. Furthermore, highly charged liposomes are beneficial in preventing spontaneous fusion before applying the molecular glue.

Enrichment of Pinolenic Acid (PLA) by Selective Ethanolysis Reaction from Pine Nut Oil with Immobilized *Candida antarctica* Lipase.

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Pine nut oil contains Δ5-unsaturated polymethylene-interrupted fatty acids (Δ5-UPIFA) in which the first double bond is in the Δ-5 position. Fatty Acid composition in the *sn*-1, *sn*-2, and *sn*-3 positions of triacylglycerol (TAG) from pine nut oil was analyzed using ethyl magnesium bromide and porcine pancreas lipase. The pinolenic acid (PLA) was predominantly located in the *sn*-3 position of triacylglycerol of pine nut oil, where they accounted for *ca.* 40 mole% of fatty acid esterified to this position. PLA was successfully isolated by lipase-catalyzed ethanolysis from pine nut oil using a commercial immobilized lipase (Novozym 435) from *Candida antarctica* as a biocatalyst. Novozym 435 in the present of ethanol showed a high regioselectivity for the *sn*-3 positions of triacylglycerol of pine nut oil. Reaction times longer than 1 h led to higher yield of PLA, but there was a concomitant steady decrease in PLA concentration in mixture of fatty acid ethyl ester.

Confirmed QTL for Soybean Protein and Oil Concentration.

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Soybean seed quality traits are quantitative traits governed by many genes. The use of molecular markers to select for genomic regions as quantitative trait loci (QTL) has reduced the time required to produce improved cultivars. The purpose of this study was to confirm soybean protein and oil QTL to facilitate molecular marker assisted selection (MAS) for soybean quality improvement. A population of 141 recombinant inbred lines (RIL) was grown in three replicates over three environments. Near infra red spectroscopy (NIR) was used to predict levels of seed protein and oil. Seventy SSR markers placed on linkage groups (LGs) B2, C2, D1a, D1b, D2, F, G, I, H, K, and M were analyzed. Seed protein QTL were confirmed on LG C2 (Satt557, Satt460, Satt079 and Satt307), LG F (Satt335, Satt114 and Satt522), LG K (Satt102 and Satt555), LG M (Satt540). Seed oil QTL were confirmed on LG C2 (Satt307 and Satt202), LG D1a (Satt436), LG D2 (Satt372), LG L (Satt076, Satt166, Satt527 and Satt561) and LG M (Satt590). This new knowledge will enable molecular breeders to further improve soybean quality.

Biotechnological Regulation of Carotenoid Pigments Overproduction by Red Yeasts.

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Carotenoid pigments represent one of the broadest groups of antioxidants. Because there has been an increased commercial interest in carotenoids as natural antioxidants and free radical scavengers in the last decade, attention has been paid on developing of suitable biotechnological techniques for their production. A range of red yeast species utilizing agroindustrial substrates were tested for carotenoids formation. Yeast strains of Rhodotorula and Sporobolomyces synthesized beta-carotene as the main pigment together with torulene and torularhodine as minor

carotenoids. In contrast, Phaffia strains accumulated astaxanthin as a principal carotenoid. Pigments biosynthesis was significantly activated when yeasts were treated by various stress conditions. In order to improve the yield of carotenoid pigments and subsequently decrease the cost of such biotechnological process, optimization of the culture conditions including both nutritional and stress factors have been applied. To more understand the basis of pigment overproduction, molecular changes in yeast cells on genome, proteome and metabolome level were studied using PAGE-SDS, 2D gel electrophoresis, LC/MS/MS and MALDI-TOF techniques. Optimized conditions in laboratory were verified in semi-scale experiments and yielded up to 35 mg beta-carotene/L and 110 mg astaxanthin/L, respectively.