2013 Annual Meeting Abstracts

Biotechnology

MONDAY

AFTERNOON

BIO 1.1/PHO 1: Polar Lipids: Chemistry, Technology, and Applications
Chair(s): X. Xu, Wilmar Global R&D Center, China; Aarhus University, Denmark; M. Ahmad, Jina Pharmaceuticals Inc., USA

Enzymatic "green" Preparation of Sugar-fatty Acid Esters

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Saccharide-fatty acid esters are an emerging category of biobased surfactants prepared entirely from renewable resources that are used as emulsifiers in foods, cosmetics, and pharmaceuticals, and possess anticancer and insecticidal properties. Typically, the esters are prepared chemically under harsh condition: temperatures near 200 C, employment of solvents, etc., which can cause undesirable side-reactions and produce waste products. Our group has been investigating the use of lipases and novel bioreactor system design to prepare sugar esters under solvent-free conditions and relatively low temperature: ~65 C. Using a stoichiometric feed of saccharide and fatty acid, our approach achieves 90-95% pure ester on a 10-30 gram scale, which, due to the absence of excess reactants and solvent, will require little or no further downstream purification to achieve industrial specifications. The presentation will provide an overview of our recent work, including an evaluation of its physical properties.

Deep Eutectic Solvents: new Opportunities for Lipase-catalyzed Reactions

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In recent years, researchers focused on finding green alternative media to organic solvents for enzyme-catalyzed reactions. Thereby, ionic liquids (IL) emerged as fascinating media for biotransformation. However, one drawback to their wider development is their cost, synthesis and purification. Recently, a novel medium with similar properties to IL but with additional advantages regarding cost, environmental impact and synthesis has been created: Deep Eutectic Solvents (DESs). DESs result from the association between an ammonium or phosphonium salt with a hydrogen-bond donor. Results showed the superior performance of choline chloride pair with urea or glycerol over other types of DESs in improving the conversion and selectivity of alcoholysis reaction of aliphatic ester using Candida antarctica B lipase (iCALB). We demonstrated that some DESs can react and compete with the substrates in alcoholysis reactions leading to byproduct formation and DES destruction. Although we know that iCALB denaturates in solutions of urea, it did not denaturate quickly in DESs containing urea or glycerol and its stability is sufficient to allow the reaction for several days. Finally, we opened new perspectives on the enzymatic modification of polar subtrate with this new generation of green, cheaper and easy to handle solvent in binary mixture with water.

Highly Efficient Synthesis of Phosphatidylserine in a Novel Medium

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Recent reports have shown that phosphatidylserine (PS) supplemented in the diets play an important role in preventing Alzheimer's dementia, improving memory, increasing attention and relieving depression. PS synthesis via enzyme-mediated transphosphatidylation of phosphatidylcholine (PC) with L-serine has been gaining more attention due to mild reaction conditions and environmental friendliness. Generally, this reaction is carried out in a biphasic system or a purely aqueous system. A serious drawback of these systems is that they contain considerable amounts of water, which results in the undesirable hydrolysis of PC and PS to form byproducts. A novel reaction system for enzymatic synthesis of PS was reported in this work. Herein, ?-valerolactone which has recently been described as an excellent candidate of green solvent available naturally was employed as the reaction-medium. Our results indicated that, under the optimized reaction conditions (i.e.: 40°C; substrate molar ratio (L-serine/PC) 3; 60 U Phospholipase D from Streptomyces chromofuscus; 12 h), PS yield could be achieved to 95% combined with no byproduct formation. In particular, the present work accommodated a facilely and efficiently enzymatic strategy for preparing PS, which possessed obvious advantages over the reported processes in terms of high efficiency.

Role of phospholipid in formation of nanoemulsions of bioactive lipids

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Bioactive lipids are defined as changes in lipid moities that result in functional consequences. Their bioactivity makes them very sensitive natural deterioration and so the use and delivery system of bioactive lipids should be critically planned so as to obtain the full benefits of it. Presently, Nanoemulsion-based delivery systems represent an effective approach to improve the dispersion of the bioactives into food products, to protect them against degradation or interaction with other ingredients, to reduce the impact on organoleptic properties of the food and to improve their bioavailability. The formation of very fine emulsions in the nanometric range (< 200 nm) can be achieved by high pressure homogenization at low temperature in presence of a suitable emulsifier which can increase its kinetic stability and minimise the impact on the organoleptic properties. In this study, an attempt was made to produce nanoemulsion of conjugated linolenic acid (CLnA) rich oil by using egg phospholipid as emulsifier; as phospholipid and CLnA can show synergistic protective effect in human system. The two forms of phospholipids were used as emulsifiers, phospholipid and lysophospholipid, and the efficiency of the two emulsifiers to prepare nano-emulsions was analyzed. The droplet size and zeta potential of the two types of nanoemulsions were determined after just preparation of emulsions and after one month of storage.

Microencapsulation of Krill oil Using Complex Coacervation

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The research work was aimed at the optimization of a bioprocess to yield gelatin/gum arabic multinuclear

microcapsules of krill oil (KO), via complex coacervation. Initial screening experimental work was performed to identify the parameters that have the most significant effects, including the homogenization speed, ratio of core to wall materials (RCW), concentration of wall materials (CWM), pH and stirring speed on the encapsulation efficiency (EE) of KO. On the basis of the results of the screening trials, a three-level-by-three factor Box-Behnken design was used to evaluate the linear, quadratic and bilinear effects of RCW (1.25:1 to 1.75:1), pH (3.8 to 4.2) and stirring speed (2 to 4, over a scale of 10) on the EE of KO. The optimal predicted conditions for the microencapsulation of KO, were RCW (1.75:1), pH 4 and stirring speed of 2, with a 90.6% of EE. The chromogenic red-orange color of KO, conferred by astaxanthin, facilitated the stereomicroscopic visualization of the entrapped oil and without the need of a lipid-soluble dye. The microcapsules, formed by complex coacervation, were circular in shape and had sufficient stability to maintain their structure, in the absence of any cross-linking agent.

Improved Acylation of Phytosterols Catalyzed by Candida Antarctica Lipase a With Superior Catalytic Activity

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Technical processes of chemical esterification presently used for the preparation of steryl esters are generally performed at high temperature in the presence of chemical catalyst, accompanying with high energy consumption, browning of products and low selectivity. This work reported a novel approach to synthesize phytosterol (?-sitosterol as a model) fatty acid esters by employing immobilized CAL A which shows a superior catalytic activity to other immobilized lipases including CAL B, Lipozyme NS-40044 TLL and Lipozyme TL IM. CAL A achieves 6-14 times faster esterification of ?-sitosterol with myristic acid than other lipases. The effects of enzyme concentration, fatty acid types, substrate molar ratio, reaction temperature and time, and polar/non-polar organic solvents were investigated. A series of ?-sitosteryl fatty acid esters (C2-C18) have been successfully prepared with structural identification of products by 1HNMR and Fourier transform-infrared spectroscopy (FTIR). CAL A showed low activity towards short chain fatty acids (C2-C6) but it increased significantly in the presence of fatty acid anhydride counterparts. CAL A rendered remarkably high activity for medium and long chain fatty acids (? C8). An increase in double bond in fatty acid molecule reduced the esterification activity of CAL A. Reaction time, temperature, enzyme load, substrate ratio and concentration, and solvent property are found to profoundly influence reaction rates. 93-98%Yield of ?-sitosteryl esters could be achieved with hexane as solvent, fatty acid (C8-C18)/ ?-sitosterol (1:1, mol:mol), 5-10% CALA load at 40-50°C for 24h. This work demonstrated the promising potential of CAL A in bioprocess of phytosterols for value-added application.

BIO 1: Enzyme Processes Enable High Yield Biodiesel Production

Chair(s): H.C. Holm, Novozymes A/S, Denmark; R. Burton, Marc-IV, USA

The Birth, Infancy and Extended Adolescence of Enzymatic Catalysis as a Technology for Industrial Biodiesel Production.

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Lipases: optimized by nature for reactions at the carboxylic acid group of fatty acids; likely produced by every living organism; known to science for over a century; conceivably capable of producing the fatty acid esters that constitute biodiesel with reduced energy input, reduced waste streams and cleaner products than traditional chemical catalysis. It would seem that their application for biodiesel production would be both a high priority and a straightforward task. Indeed, to those in the biodiesel sector it may appear that the technology for their use in this application burst fully

formed on the industry within the past two years. However, the first studies of enzyme-catalyzed biodiesel production are now nearly 30 years old, and the size of the effort expended in pursuit of this technology is attested to by the fact that there are more than 4000 primary research publications on the topic. Advances and success in this area have taken insight, innovation, technology development and inputs from individuals with skills in a diversity of areas, some quite removed from applied enzymology. The purpose of this presentation is to overview the history of the development of enzymatic biodiesel production in order to foster an understanding and appreciation of the successes embodied in the subsequent talks in this session.

Commercial Biodiesel Production Using Enzyme Catalysis

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In commercial biodiesel production, the ability to process a wide range of feedstock grades provides favorable economics to the producer. Often lower cost feedstocks contain high levels of free fatty acids (FFA) and because of this are difficult to process into biodiesel. The traditional chemical means of processing high FFA feedstocks, acid-catalyzed esterification, requires the use of sulfuric acid and high proportions of methanol to fatty acid. In addition, the equipment for recovery and purification of the side streams from such processes requires a high capital investment and a high energy input. It is now proven that the immobilized or liquid phase enzymes can be utilized for the production of biodiesel. The use of these enzymes in the processing of fatty acid esters can reduce waste alcohol streams, reduce energy input, increase production yield, and enhance the co-product quality of glycerol. This paper will evaluate the commercial experiences of an enzymatic process operating in a biodiesel production facility.

A Flexible Modular Process Design for Enzymatic Biodiesel Production

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The increasing academic and industrial interest in biocatalytic processes (catalyzed by an isolated enzyme, immobilized enzyme or whole-cells containing one or more enzymes) is driven in large part by the need for selective chemistry (with few side reactions, under mild conditions). Such selectivity also drives the application of lipases of various types to catalyze primarily esterification and transesterification reactions to produce biodiesel. Clearly the yield of biodiesel and effective use of the enzyme(s) is important for economic operation. However of equal importance is achieving a product which is within specification. A combination of separation technologies together with different combinations of enzyme steps will be required in order to achieve this, dependent upon the feedstock. For maximum flexibility it will be important to have the possibility to use differing feedstock oils of varying quality. In this presentation we will present the concept of a generic process plant where a range of feedstocks can be used in the same plant, via flexible modular process design. While the capital required for such a plant is greater, there is little doubt that the opportunity to use a range of feedstocks will pay back. Data from our laboratory and pilot plant scale studies will be used to support the design calculations.

Technical Aspects of the High Purity Glycerin Phase from Enzymatic Biodiesel Production

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Retrofitting a chemical biodiesel plant to enzymatic catalyzed biodiesel, and operational experience

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The development in oil prices has forced the biodiesel industry to think of new way of optimizing the production and to utilize cheaper raw materials. The lower cost feedstocks come with disadvantages as for instance high FFA which the alkaline catalyzed process cannot process right away. An interesting alternative to this process has been developed during the last couple of years by Novozymes. We have evaluated the economy of the process and found it cost effective to implement at a major biodiesel producer in the central United States. The presentation will discuss the experience from retrofitting the plant to the Novozymes BioFAME process and the experiences gathered from operating the enzyme process in the plant with different oils feedstocks.

Progress of Lipase-catalyzed Biodiesel Commercialization

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Lipase-catalyzed transesterification from renewable oils for biodiesel production has many advantages over chemical ways. However, the low stability and the high cost of lipase have been thought to be the main hurdle to the industrialization of lipase-catalyzed biodiesel production. Tsinghua University has proposed a novel route for biodiesel preparation, which can reduce the lipase cost dramatically. This novel process is promising for the commercialization of biodiesel production. This presentation will present the updated progress of this enzyme-mediated biodiesel production on industrial scale and pilot scale in China and Brazil, respectively. As a by-product, glycerol will be produced at about 10% of biodiesel during the process of biodiesel production. How to convert glycerol has become a common problem which has to be resolved if considering large amount of biodiesel production. Integrated production of 1,3-propanediol (PDO) from glycerol could be a promising way to improve the profit of the whole process during biodiesel production. 1,3-PDO is a valuable chemical material and especially it can 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 process for 1,3-PDO production from glycerol. Currently the large-scale production of 1,3-PDO(20000tons/year) is being under construction.

How enzymes are utilized for the design of a cost efficient biodiesel process

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The biodiesel industry has increasingly being using lower cost oil feedstocks to be cost effective. These oils can be difficult to process in a traditional biodiesel plant, e.g. due to the FFA in the oil. The enzymatic catalyzed biodiesel process ? Novozymes BioFAME process ? is handling any content of FFA, which gives a large flexibility in the raw material supply. As the enzyme reaction does not cause salt formation the glycerin phase is very pure. it is possible to use a liquid formulation of lipase which can also be re-used. The necessary number of re-uses of the liquid lipase is

much lower than for immobilized enzymes due to the large difference in the price of the enzymes. The change to transesterification with liquid instead of immobilized enzyme has resulted in a significant simplification of the process and is the background for the cost efficiency of the process. The process is catalyzed by liquid formulation of lipase and the stability of the enzyme is important as we need to re-use it for several batches. After the reaction TG and DG is within ASTM specifications (i.e. < 0.2%), MG approx. 1% and FFA approx 2%. The MG and FFA are brought within specifications by a caustic washing step and recovered for FAME production in a later batch. This secures a very high yield. There are alternatives to the caustic wash for ?polishing? of the biodiesel. For example enzymatic esterification is preferred by some producers although the overall production costs can be slightly higher.

Industrial Enzymatic Production of Biodiesel Fuel

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Utilization of lipases for the production of fatty acid methyl esters, typically used as biodiesel fuel, has been intensively studied during the last decade. Most widely applied lipases for this task included those derived from Candida antarctica, Burkholderia cepacia, Thermomyces lanuginose, Pseudomonas fluorescens, Alcaligenes sp., Candida rugosa, and Mucor miehei. Lipases mostly in their native form mixed either in polysaccharide-based excipients or dissolved in a liquid polyol, have been used to catalyze transesterification/esterification reactions to form fatty acid methyl esters from different oil feedstocks and methanol. Alternatively, lipases extracted from such microorganisms have been immobilized on different enzyme carriers and used either in stirred tank- or fixed-bed reactors to catalyze transesterification/esterification reactions for the production of biodiesel. This study has been focused on the use of new immobilized lipases of different substrate selectivity to produce biodiesel fuel complying with the ASTM and EN Specs. Based on results obtained in lab scale models, different industrial scale systems have been developed for the production of biodiesel fuel and its by-products glycerol and water. The whole system is comprised of three basic units: 1) Feedstock pre-treatment unit, 2) Enzyme reactor, and 3) Product post-treatment unit.

TUESDAY

MORNING

BIO 2: Biocatalysis I
Chair(s): J. Ogawa, Kyoto University, Japan; C.T. Hou, USDA, ARS, NCAUR, USA

Cloning and Heterologous Expression of Glycosyltransferase for Glycolipid and Sterylglucoside Synthesis

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Candida bombicola is widely studied for sophorolipid production. Its ability to synthesize other glycolipids, however, had not been previously reported. In this paper, we describe for the first time the gene-cloning and characterization of a unique glycosyltransferase (GT) from C. bombicola chromosome. GT was cloned by PCR chromosomal walking strategy from C. bombicola. It was then expressed in common baker?s yeast Saccharomyces cerevisiae. We demonstrated that the extracts of the recombinant S. cerevisiae could catalyze in vitro synthesis of sterylglucoside and

fatty acid-glucoside as identified by LC/MS. Current research centers on developing this recombinant S. cerevisiae and the original C. bombicola for the fermentative production of glycolipid and sterylglucoside useful as biosurfactant and nutraceutical products.

Functional Lipids From Fermented Marine Products

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We developed a novel seafood paste from scallop eggs by fermentation with rice malts, salts and yeasts. The product contained a large amount of omega-3 PUFAs, mainly EPA and DHA. Although the fermented paste contained high level of omega-3 PUFAs, little lipid oxidation has been found during the storage for two month at 37 oC. The high oxidative stability of fermented paste lipids was mainly due to polyphenols contained in rice malts, scallop egg carotenoid, pectenolon, and peptides formed during fermentation. The fermentation process produced monoesters of PUFAs and the contents increased with increasing fermentation time. Luciferase reporter assay showed that these monoesters strongly activated PPAR gamma expression, which has been known to be a key bio-molecule for lipid metabolism. Overall, fermented scallop egg product can be used as a new functional food containing a high level of omega-3 PUFAs.

Dual Production of Polyunsaturated Fatty Acids and Pigments by Fungi

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There is an increasing demand for high-valued and biologically active compounds from natural sources, such as polyunsaturated fatty acids (PUFAs) and carotenoid pigments. Unfortunately, inadequacy of natural sources riched in PUFAs and pigments do not allow them to be used in large quantities as food/feed supplements. However, PUFAs and carotenoids have also been found in diverse microorganisms. Of them, zygomycetous fungi offer challenging potential for biotechnological production of PUFAs with dual biosynthesis of carotenes (?-carotene, ?-carotene and lycopene). Although these fungi could be used for large scale submerged and solid state fermentations, different physiological strategies have to be applied to optimize the yield of either PUFA or beta-carotene in the final microbial product. Especially, fungal solid state fermentations allow effective valorization and transformation of many agroindustrial materials to various types of value-added bioproducts enriched with these bioactive compounds. Prefermented products obtained after solid state fermentations of cereals by fungi are characterized with high ?-linolenic acid (2.4 g/kg) and ?-carotene (8.5 mg/kg) content. Such products with demanded nutritive, functional and flavor value, improved antioxidant, radical scavenging and thermooxidation properties and with enhanced safety (removing anti-nutrients, hydrolyzing biopolymers) have been successfully applied as inexpensive functional food and feed supplements. The work was supported by grant VEGA 1/0975/12 and by grants APVV-0662-11 and APVV-0294-11.

Screening and Application of Microbial Oxidase and Oxygenase

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Microbial oxidizing enzymes including oxidases and oxygenases have vast potentials as biocatalysts in bioprocess development and in environmental biotechnology. Here, we introduce our current researches on laccase as a novel bio-controlling agent and on alpha-ketoglutarate-dependent dioxygenases as a fine chemical catalyst. Laccase catalyzes the oxidation of a wide range of inorganic and aromatic substances (particularly phenols) with the concomitant reduction of O2 to H2O. The concerned action of laccases and oxidizable low-molecular-weight compounds, so called mediators, was found to extend or permit oxidation of nonsubstrates. We investigated whether laccase-mediator system can be used as bio-controlling agents replacing conventional agricultural chemicals. The anti-microbial effects of laccase-mediator system were observed towards several plant pathogens including filamentous fungi, actionomycetes, and bacteria. Alpha-ketoglutarate-dependent dioxygenases are useful for the production of hydroxyl? amino acids. We developed practical bioprocess system of 4-hydroxyisoleucine, which had remarkable anti-diabetic activity, by using an alpha-ketoglutarate dependent?dioxygenase (IDO) derived from Bacillus thuringiensis 2e2 as a biocatalyst. Then it was found that IDO and two dioxygenases, derived from Nostoc punctiforme and Burkholderia ambifaria, were good biocatalysts for production of various amino acid derivatives.

Function of Novel Enzymes Involved in Conjugated Linoleic Acid Production From Linoleic Acid in Lactic Acid Bacteria

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Conjugated fatty acids have attracted much attention as a novel type of biologically beneficial functional lipid. In particular, the unique activities of conjugated linoleic acid (CLA) have been intensively studied, showing that CLA is expected to be a potential material for pharmaceuticals and dietary supplements. Natural sources of CLA are dairy products from ruminants, although CLA contents are very low. Furthermore, the mechanism of CLA production was not revealed clearly. We screened the ability to produce CLA from linoleic acid within lactic acid bacteria, and selected Lactobacillus plantarum AKU 1009a as a potential strain. This strain was found to transform the cis-9,cis-12 diene structure of C18 fatty acids such as linoleic acid, ?-linolenic acid, and ?-linolenic acid into conjugated diene structures of cis-9,trans-11 and trans-9,trans-11 with 10-hydroxy- fatty acids as intermediates. We analyzed the CLA-producing pathway in this strain, and found that this pathway included a novel multi-component enzyme system that consists of three enzymes, CLA-HY, CLA-DH, and CLA-DC. We tried to identify the function of these enzymes and found that CLA-HY catalyzed hydration/dehydration reaction, CLA-DH catalyzed oxidation/reduction reaction, and CLA-DC catalyzed isomerization reaction. In this study, we will introduce these reactions in more detail.

Real-time Small-angle Neutron Scattering Analysis of Lipase-catalyzed Biodiesel Production in Microemulsion Systems

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Lipase-catalyzed synthesis of biodiesel is potentially an attractive alternative to alkali-catalyzed transesterification for several reasons: utilizion of free fatty acid (FFA)-rich feedstocks and employment of safer and more sustainable synthesis conditions (lower stoichiometric excess of alcohol, energy cost, and downstream purification requirements, and waste product production. Fundamental research is required to elucidate the partitioning behavior of reactants, products, and intermediates, and its relationship with the observed kinetics, in order to improve the reaction rate and yield (e.g., prevent product inhibition) since this reaction occurs at the liquid-liquid interface in all situations. To

achieve this goal, the lipase-catalyzed esterification of lauric acid and 1-butanol has been conducted in water-in-oil microemulsions: a thermodynamically stable solution of aqueous nanodro;plets dispersed in apolar media through the addition of surfactant (Aerosol-OT). The reaction has been monitored using small-angle neutron scattering (SANS) to ascertain (perhaps quantitatively) the effect of partitioning during the time course of reaction, achieved through selective deuteration of media components to acheive a difference in neutron contrasting across the interface. A preliminary analysis of the SANS data shows that the alcohol and fatty acid strongly partition to the interface, demonstrated by a decrease in the thickness of the interface's surfactant monolayer and of the extent of attractive interactions existing between nanodroplets, a trend that is reversed during the time course of reaction, due to the consumption of substrate.

Antibacterial Activity of Rare Unsaturated Fatty Acids Produced by Microbial Conversion

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Aeromonas hydrophila N-6 converts vegetable oils to wax esters (esters of fatty acids and fatty alcohols). The wax esters include several unsaturated fatty acids, such as 7-cis-C16:1, 5-cis-C14:1, 7-cis,10-cis-C16:2, and 5-cis,8-cis-C14:2. Since these fatty acids are rarely found in natural oils, their function has not been clarified yet. In general, unsaturated fatty acids inhibit growth of several bacteria, we thus aimed to evaluate and compare antibacterial activity of these rare unsaturated fatty acids. A. hydrophila N-6 was inoculated into culture medium with 3 kinds of triacylglycerol composed of oleic acid (9-cis-C18:1) or linoleic acid (9-cis,12-cis-C18:2) or ?-linolenic acid (9-cis,12-cis,15-cis-C18:3), and cultivated for 5 days. By the chloroform/methanol extraction, silica gel chromatography, chemical hydrolysis, solvent fractionation, and HPLC fractionation, 7-cis-C16:1, 5-cis-C14:1, 7-cis-C14:1, 7-cis,10-cis-C16:2, 5-cis,8-cis-C14:2, 7-cis,10-cis,13-cis-C16:3, and 5-cis,8-cis,11-cis-C14:3 fatty acids were purified. 9-Cis-C18:1 showed weak antibacterial activity against Staphylococcus aureus NBRC13276 (minimum inhibitory concentration, MIC, 630 ?g/mL). In contrast, 7-cis-C16:1 fatty acid showed the strongest activity (MIC, 8.8 ?g/mL) among the tested 29 fatty acids. This antibacterial activity was stronger than the activity of palmitoleic acid (9-cis-C16:1, MIC, 19.2 ?g/mL) observed in several natural oils. Furthermore, we clarified that the carbon chain length and double bond position of the fatty acids were important for the antibacterial activity against S. aureus NBRC13276 and Staphylococcus epidermidis NBRC100911.

Monocyclic Carotenoid Produced by Marine Bacteria

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Carotenoids are red, orange and yellow color pigments composed of isoprenyl units. They are well known to have beneficial health effects including anti-oxidant activity, anti-cardiovascular disease and anti-cancer effects. By screening marine microbes, several pigmented bacteria which produce yellow, orange and red pigments were separated. Among them, 11ShimoA1 strain which was identified to Jejuia pallidilutea produced light orange pigment different from the color of zeaxanthin and astaxanthin. In addition, we also isolated Persicivirga ulvanivorans produced another orange pigment from sea water. As the result of NMR analysis, pigments produced by strain 11ShimoA1 were identified to a novel monocyclic carotenoid, 2'-(3-methylbut-2-enyl)-3',4'-didehydro-1',2'-dihydro-?,?-carotene-3,1'-diol (2?-isopentenyl-saproxanthin) and zeaxanthin. Further, an orange pigment produced by Persicivirga ulvanivorans was myxol (3?,4?-didehydro-1?,2?-dihydro-?,?-carotene- 3,1?,2?-triol). The production of 2?-isopentenyl-saproxanthin was increased by the incubation in the Marine Broth 2216 adjusted at pH 9.2. Further, blue LED

stimulation increased production of 2?-isopentenyl-saproxanthin, but not zeaxanthin, by 11SimoA1.

Transformation of Cereals by Synthetic Gene Encoding for Delta-6-desaturase

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An economical importance of cereals can be increased by improving quantitative as well as qualitative trait such as nutritional value. Beside polysaccharides, cereals are the main source of proteins in human nutrition, but there is a lack of n-3 and n-6 polyunsaturated fatty acids. Changes in the composition of fatty acids in cereal grains are not feasible by classical breeding methods, but an alternative approach to achieve this task might be based on aimed genetic transformation of cereals. Delta-6-desaturase (D6D) is an enzyme catalyzing conversion of linoleic acid (C 18:2, n-6; LA) to gamma-linolenic acid (C18:3, n-6, GLA). Synthetic D6D gene was prepared with codon usage optimized for cereals. This synthetic gene was cloned into vector with endosperm specific promoter Dx5 (gene encoding subunit of high-molecular-weight glutenin) and signal sequence of Dx5 gene was inserted in front of D6D gene with the aim to destinate fused protein into endoplasmic reticulum (PUFA synthesis location). Immature scutella of cereals were transformed by biolistic method. Transgenic plants were confirmed at genomic, transcriptomic and metabolomic level and GLA as the product of enzymatic reaction was confirmed using GC/MS. Moreover, stearidonic acid (C18:4, n-3, SDA), that is biosynthesized from alpha-linolenic acid by D6D, was also detected in transgenic cereals. It should be emphasized that synthesis of neither GLA nor SDA in cereal grains has never been described so far. The work was supported by grants APVV-0294-11 and APVV-0662-11.

AFTERNOON

BIO 3: Biocatalysis II

Chair(s): C.T. Hou, USDA, ARS, NCAUR, USA; S.H. Yoon, Korea Food Research Institute, South Korea

Structured Lipid Synthesis by Enzymatic Transesterification

S. Yoon[(1)], J. Rhee[(2)]

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Salatrim (short and long acyl triglyceride molecule) is a family of structured lipids that provides the physical properties of fat, but with approximately half of the calories of typical edible oil. If the stearic acid is at the 2-position of the triacylglycerols, the resulting 2-monostearin is steadily absorbed. If, however, it is at the 1- or 3-position, it is released as free stearic acid and, in the presence of calcium and magnesium, it is poorly absorbed. Thus, the position of long-chain fatty acid in the triacylglycerol molecule is very important for calories. Enzymatic methods need small energy consumption compared to chemical methods and can remove side-product production. For the production of salatrim, stearic acid and acetic acid were chosen for their fatty acid composition. Short-chain fatty acid such as acetic acid gives low caloric availability and long-chain fatty acid (more than C18) such as stearic acid is seldom absorbed by the body. Total caloric availability of salatrim is lower than that of the typical edible oil. Long chain fatty acids could be incorporated mainly at the 1- or 3-position of the triacylglycerols by 1,3-specific lipase and, thus, the caloric

availability of the salatrim synthesized by the enzymatic reaction would be lower than that synthesized by the chemical reaction. Transesterification reaction with triacetin and stearic acid using lipase in a solvent-free system for the production of salatrim were performed and characterized.

Production of Punicic Acid in Arabidopsis Seed Oil

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Punicic acid (18:3?9cis,11trans,13cis) is a conjugated linolenic acid isomer which is major component of Punica granatum (pomegranate) seed oil. Punicic acid has been shown to have both anti-cancer and anti-obesity properties. Development of oilseed crops producing high levels of punicic acid could provide for a cheaper and more readily available source of this nutraceutical fatty acid. Fatty acid conjugase (FADX; AY178446) catalyzes the conversion of linoleic acid (18:2?9cis,12cis) into punicic acid. This enzyme is a homolog of FAD2 which catalyzes the formation of linoleic acid from oleic acid (18:1?9cis). Previous expression of a cDNA encoding FADX, during seed development, in Arabidopsis thaliana resulted in accumulation of punicic acid up to 3.5% (wt/wt) of the fatty acid chains in the seed oil (Iwabuschi et al., 2003, J. Biol. Chem. 278:4603-4610). Production of punicic acid, however, was accompanied by increased accumulation of oleic acid, which suggested that that FAD2 activity was inhibited in the transgenic plants. In the current study, we expressed P. granatum FADX and FAD2 (AY178447), during seed development, in a fad3/fae (fatty acid elongase) 1 mutant of Arabidopsis which is enriched in linoleic acid. Some transgenic lines produced through this approach had levels of punicic acid greater than 20% of the fatty acid chains in the seed oil. The results of our analyses of these transgenic lines will be presented.

Fractionation of Conjugated Linoleic Acid Isomers by Lipase Reactions

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[(1)]The Nisshin OilliO Group, Ltd., Japan

Conjugated linoleic acid (CLA) is industrially produced by alkali conjugation of linoleic acid-rich oils, in the presence of propylenglycol. The product consists of almost same amounts of the isomers, 9-cis,11-trans-CLA (c9t11) and 10-trans,12-cis-CLA (t10c12), which exhibits various physiological properties, such as reduction of cancer incidence, beneficial effects in atherosclerosis, decrease in body fat content, and improvement of immune functions. In addition, the two isomers have been reported to show different activities: c9t11 isomer exhibits anti-tumor activity, and t10c12 isomer decreases body fat, increases energy expenditure, and suppresses the development of hypertension. To purify the two isomers, we developed two step processes using a lipase. 1. To synthesize tri-conjugated-linoleoylglycero (CLA-TAG) smoothly, we used the different lipases powder which have different characteristic. The rate of synthesis of CLA-TAG depended on the blend ratio of the lipase powder. In the case of using only one lipase powder, the rate of synthesis was seriously decreased. 2. To purify the two isomers, CLA-TAG was selectively hydrolyzed by using C. rugosa lipase which acted more strongly on c9t11 than t10c12. Consequently, c9t11-CLA is enriched in the FFA fraction and t10c12-CLA is enriched in undigested the acylglycerol fraction. The t10c12 enriched acylglycerols can be changed to FFA by non-selective hydrolysis.

Fatty acid hydratases and isomerases: can we understand their catalytic promiscuity?

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Fatty acid hydratases catalyze the addition of water to double bonds forming e.g. ricinoleic acid whereas isomerases catalyze the migration of a double bond to form e.g. conjugated linoleic acid. Based on the structural and mechanistic similarities and differences between these enzymes we developed a strategy to alter their catalytic properties with the aim to understand their catalytic promiscuity and evolutionary relationship.

Enzymatic preparation of chiral intermediates for development of drugs by lipases

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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 over expressed 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 enzymatic preparation of chiral intermediates for the synthesis drugs by lipases.

Formulation and Characterization of Trans-free Margarine Containing Stearidonic Acid

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Omega-3 fatty acids (n-3 FAs) have been positively associated with prevention and treatment of chronic diseases. Intake of high amounts of trans fatty acids (TFAs) is correlated with increased risk of coronary heart disease, inflammation, and cancer. Structured lipid (SL) was synthesized using stearidonic acid (SDA) soybean oil and high stearate soybean oil catalyzed by Lipozyme® TLIM lipase. The SL was compared to extracted fat (EF) from a commercial brand for FA profile, sn-2 positional FAs, triacylglycerol (TAG) profile, polymorphism, thermal behavior, oxidative stability, and solid fat content (SFC). Both SL and EF had similar saturated FA (~31 mol%) and unsaturated FA (~68 mol%), but SL had a much lower n-6/n-3 ratio (1.1) than EF (5.8). SL had 10.5 mol% SDA. After short-path distillation, a loss of 53.9% was observed in the total tocopherol content of SL. SL and EF had similar melting profile, ?' polymorph, and oxidative stability. Margarine was formulated using SL (SLM) and EF (RCM, reformulated commercial margarine). No sensory difference was observed between the two margarines. The SL synthesized in this study contained no TFA and possessed desirable polymorphism, thermal properties, and SFC for formulation of soft margarine. Our results suggested that the interesterified product could be used as an alternative to partially hydrogenated fat without trans fat. The experimental margarine was enriched with plant-based n-3 FAs and comparable to commercial margarine, thereby increasing the food applications of SDA soybean oil.

Characterization of Acyl-coa Synthetase Genes From Oleaginous Fungus *mortierella Alpina* 1s-4

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Filamentous fungus *Mortierella alpina* 1S-4 produces triacylglycerols rich in arachidonic acid (ARA). The microbial lipid production reaches 20 g/L with 30-70% ARA in the total fatty acids. A multiple transformation system for *M. alpina* 1S-4 and its derivative mutants allowed manipulation of genes involved in polyunsaturated fatty acid (PUFA) biosynthesis for improvement of the production of various PUFAs. On overexpression or RNA interference of endogenous fatty acid desaturase or fatty acid elongase genes, the fatty acid compositions in the transformants from *M. alpina* 1S-4 were modified. It is of great interest to elucidate the mechanisms in accumulation of a large amount of lipids in mycelia, and the physiological function of lipid accumulation and ARA biosynthesis in *M. alpina* 1S-4. *M. alpina* 1S-4 has some homologue enzymes involved in lipid biosynthesis in some cases. Homologous genes encoding twelve acyl-CoA synthetases (ACS) were found in *M. alpina* 1S-4, which catalyze the conversion of a free fatty acid to an acyl-CoA. An acyl-CoA unit plays an important role in biosynthesis and conversion of lipids. In this research, we assessed the effects of overexpression of ACS genes in *M. alpina* 1S-4 with aiming at the enhancement of lipid productivity by molecular breeding, and evaluated substrate specificities of some fatty acids for the ACS genes.

Useful Polyunsaturated Fatty Acid Production by Oleaginous Filamentous Fungus *mortierella Alpina* Breeding

A. Ando[(1)]
[(1)]Kyoto University, Japan

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

WEDNESDAY

MORNING

BIO 4/S&D 4: Biobased Surfactants, Detergents and Oleochemicals
Chair(s): G. Smith, Huntsman Performance Products, USA; D. Hayes, University of Tennessee, USA; D. Solaiman, USDA, ARS, ERRC, USA

Vegetable Oil Based Surfactants: Physical Chemistry and Performance Properties

G. Smith[(1)]
[(1)]Huntsman Corporation, United States of America

Vegetable Oil Based Surfactants: Physical Chemistry and Performance Properties Modern surfactants are based on either naturally derived or synthetic feedstocks. Natural surfactants are typically based on alcohols derived from coconut or palm kernel oil whereas synthetic surfactants are based on ethylene derived from gas, oil and coal. Synthetic surfactants are not based on renewable feeds and in recent years there has been increased demand for more sustainable alternatives. While natural alcohol based surfactants based on coconut or palm kernel are more sustainable, there has been increased concern about destruction of the rain forest and resulting loss of biodiversity. This presentation will discuss the physical chemical properties of surfactants based on vegetable oils like soy and canola as low cost, locally grown alternatives to conventional natural and synthetic based surfactants. A series of vegetable oil based surfactants were prepared by reacting different natural oils like soy and canola with different polyols. Depending on the polyol employed, different types of surfactants can be prepared. Reacting ethoxylated glycerin with the triglyceride gives vegetable oil ethoxylates (VOE). The properties of vegetable oil derived surfactants have been compared to more conventional natural alcohol ethoxylates (AE). In general, vegetable oil surfactants have a lower CMC, cloud point and foam potential than AEs due to the longer alkyl chain length. Surface and interfacial tension depend on the alkyl chain distribution and the degree of polymerization on the polyol. Vegetable oil derived surfactants show good detergency in single surfactant and multi-component systems.

Biobased Surfactants: Overview and New Directions

D. Hayes[(1)]
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Biobased surfactants continue to gain popularity due to concerns for sustainability and the long-term availability of petrochemical feedstocks. In this presentation, market information and overall trends for biobased surfactants will be reviewed, with major commercially available biobased surfactants identified. New biobased surfactants under development, as described in recent Journal of Surfactants and Detergents publications, will be described.

Sophorolipids as Antimicrobials and as Composite Additives for Phenotypic Alteration of Polyhydroxyalkanoate Film Surfaces

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Sophorolipids are microbially-based glycolipids that are synthesized in high yields from certain species of Candida yeasts. Because of their chemical structure, which normally consists of sophorose (2?-O-beta-glucopyranosyl-beta-D-glucopyranose) and a C16 or C18 fatty acid tail, sophorolipids are slowly being adopted industrially as additives in detergent and other cleaning applications. However, fermentative yields of greater than 100 g/L have stimulated the search for new, novel applications for these molecules. In this presentation we will discuss our efforts in demonstrating the use of sophorolipids as antimicrobial compounds aimed specifically at the acne-causing bacterium Propionibacterium acnes and show how different biopolymer matrices can be used to enhance the bacteriocidal effects of sophorolipids. We will also show the results of our work in utilizing sophorolipids as additives to various polyhydroxyalkanoate (PHA) biofilms. Scanning electron microscopy revealed that sophorolipids produce a controllable ?dimpling effect? in PHA biofilms which alter the material properties of the films. By varying the amount of sophorolipid that is introduced to the PHA films the number and size of the dimples as well as the porosity created within the film matrix can be controlled which may result in potentially unexplored applications in areas such as tissue scaffolding and bioremediation.

Biological activities of rhamnolipids and their incorporation and release from hydrogel formulations

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The main focus of this presentation is on the incorporation and release of rhamnolipids from several hydrogel formulations that we have been developing. In addition to these observations and results, the following topics will be included: 1. Production of different mixtures of mono- and di-rhamnolipids, 2. Methods for purification of rhamnolipids and separation of their mono- and di-rhamnolipid components, and 3. Biological activities of rhamnolipids on organisms.

Enzymatic synthesis, surface and lipid interaction properties of novel rhamnolipids

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Amongst glycolipidid biosurfactants, rhamnolipids have drawn particular attention as they have several interesting biological properties such as antimicrobial, antiphytoviral, zoosporicidal and plant defense elicitor activities [1-3]. It is generally recognised that these activities must be linked to the interaction of these molecules with constituents of biological membranes [4] but the detailed mechanism is far from being fully understood. The objective of this work is double. First, it aims to investigate a new strategy of synthesis for the production of novel rhamnolipids [5] that could exhibit properties as promising for industrial and environmental applications as their natural counterparts while avoiding the use of the pathogenic Pseudomonas aeruginosa for their production. Secondly, their basic surface properties (critical aggregation concentration, surface tension at CAC and interfacial behaviour of their monolayer) and their interaction with model membranes are investigated in relation with their structure in order to give insight about the mechanism of their biological actions. [1] Vatsa P. et al. Int. J. Mol. Sci. 2010;11:5095. [2] Varnier A-L. et al. Plant, Cell Environ. 2009;32:178. [3] Lang S. et al. Appl. Microbiol. Biotechn. 1999;51:22. [4] Aranda F.J. et al. Langmuir. 2007;23:2700. [5] Nott K. et al. Process Biochemistry, http://dx.doi.org/10.1016/j.procbio.2012.11.019

Drug Delivery Systems based on diacyl arginine surfactants: preparation, characterization and evaluation of their biological activity

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In the last years our group has prepared new diacyl cationic surfactants based on the amino acid arginine, with different structures (gemini, and glycerolipid), characterized by relevant nontoxic and antimicrobial properties as well as rapid biodegradability. In addition, these surfactants from arginine are extraordinarily active in reducing surface tension. Cationic colloidal systems composed by these arginine based surfactants and membrane additive compounds have been characterized by means of size distribution and zeta-potential measurements. Gemini surfactants with the shortest spacer chain formed micelles, while aqueous solutions of pure gemini surfactants with longer spacer made up very big aggregates. The addition of phospholipids or cholesterol changed drastically the aggregation behaviour. The

capability of disrupting the erythrocyte?s membrane depends on the hydrophobicity of the molecules and the size of aggregates in the solution. The alkyl spacer chain and the presence of additives also play an important role on the antimicrobial activity. The diacyl-glycerol arginine cationic lipids form stable cationic liposomes by themselves. These formulations can encapsulate different drugs, and the percentage of encapsulated drug depends on the physicochemical properties of the vesicles as well as on the type of drug. The capacity of the systems to vehiculate different molecules was evaluated performing their in vitro drug release profiles. These results suggest that our formulations represent a great innovation in the pharmaceutical field, due to their dual pharmacological function: one related to the nature of the vehiculated drug and one related to the innate antibacterial properties of the surfactant-based carriers.

Soy Protein Fragments as Hydrophilic Components in Surfactants

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The development of protein based surfactant technology has taken many turns over the past one hundred and twenty five years. Early in the history of surfactants, protein based materials emerged as a significant technology that was developed for a number of applications. Over the years, these materials were largely replaced, first with sulfated oils and fatty acids and later with nonionic surfactants based on alkene oxide technology. By the 1950?s, surfactants based on protein technology were largely replaced with materials based on modified fats & oils, petroleum derived intermediates or the combination of the two. Since then, protein based surfactant technology and the related amino acid based surfactant technology has been confined to small volume specialty applications. Working with the United Soy Board, we are carrying out research directed at reestablishing a place in surfactant technology for soy based protein materials. We will report on the progress made in moving this technology from a research program into an early stage development program. Specifically, we will review the properties of newly developed surfactant materials and the prospects for these renewable materials finding commercial opportunities in significant applications and markets.

Eastman GEMTM technology for cosmetic ingredients

M. Natale[(1)]
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Natural ingredients have always been important in the cosmetics market, while the demand for the use of green processes is becoming more important to both formulators and consumers. While some definitions are still being debated, ?natural? typically refers to the source of the raw materials, and ?green? refers to the processes used to convert starting materials to a finished ingredient. Eastman?s GEMTM technology is centered around a green biocatalytic process to synthesize a variety of cosmetic esters via enzymatic esterifications at mild temperatures. The esterifications are driven to high conversion by removing the coproduct, usually either water from esterification of an acid or a lower alcohol from transesterification of an ester. The mild processing conditions suppress formation of undesirable byproducts that may contribute color or odor. The immobilized enzyme, such as lipase, is easily removed by filtration. The specificity of the enzymatic conversions and the relatively low reaction temperatures minimize the formation of byproducts, increase yield, and save energy. The GEMTM technology will be exemplified by the manufacture of emollient esters such as 2-ethylhexylpalmitate. The discussion will also include GEMTM processes for the preparation of active ingredients and surfactants.

Surfactants extracted from waste biomass and their use to remove oil from oil-coated sands.

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Previous presentations in this bio-based surfactant session have described the alkaline extraction of wastewater sludge to produce an alkaline solution with surface active properties that rival those of commercial surfactants. We have also described the detergency performance of these extracts and have shown that it approach that of commercial surfactants. This time we present a comparison of the ion composition and performance of alkaline extracts obtained from wastewater sludge (at the University of Toronto) and from compost (at the University of Torino). We will show that the source of biomass produces significant changes in the property of the resulting surfactant, and that the ion composition is also a factor, particularly when it comes to interfacial properties. We will discuss the low and ultralow interfacial tensions that can be obtained with various oils and mixtures of the alkaline extracts and a hydrophobic anionic surfactant, and without added salt. Finally, we will show that the mixture of alkaline waste biomass extracts and a hydrophobic surfactant is capable of improving the removal of oil from oil-coated sands. We will discuss the potential applications of this work in environmental remediation and enhanced oil recovery.

Renewable glucarate-based complexes as auto-dish builders

T. Smith[(1)]
[(1)]Rivertop Renewables, United States of America

The phasing out of phosphate in US automatic dishwashing detergents left a huge performance gap, particularly when it comes to filming and spotting of glassware. While many alternatives to-date do not meet the cost-performance levels of STPP, Rivertop Renewables has developed a high-performing, cost-effective, renewable replacement for phosphate to address the current market challenges. Rivertop has extensively studied a new builder system based on salts of glucaric acid. Glucaric acid is a novel sugar acid readily produced from glucose via Rivertop?s proprietary chemical oxidation technology. The builder system, trademarked Riose?, utilizes an aluminum glucarate complex which has excellent binding strength for calcium and magnesium. Studies have also shown that detergent formulations with Riose have high performance with respect to reduced film and spot formation. Furthermore, the builder?s glucarate component is GRAS and biodegrades naturally. Calcium chelation by the builder is pH-dependent, a property that may well apply to other metals. We refer to this performance characteristic as ?catch and release.? Calcium is caught, or chelated, by the builder in the dishwasher (pH 10+), then released at neutral pH in the wastewater plant. A future area of study will be to determine if ?catch and release? applies to other metals whose migration and concentration could be mitigated to protect freshwater supplies.

Synthesis of succinyl amide gemini surfactant from Adenopus breviflorus seed oil: A prospective corrosion inhibitor of mild steel in acidic medium for the African polpulace

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Succinyl amide gemini surfactant was synthesised using simple reaction mechanism from the seed oil of Adenopus breviflorus and applied as corrosion inhibitor of mild steel in 0.5 M HCl via weight loss method. The synthesis was monitored and confirmed using FTIR and NMR. The inhibitive mechanism of succinyl amide gemini surfactant was by adsorption which was spontaneous, exothermic and it obeyed Langmuir isotherm with the process being physisorption. The activation energy increased as concentration of succinyl amide gemini surfactant increased with the highest being 135.20 KJ mol-1 at 200 mg/L while the heat of adsorption was -95.25 KJ mol-1 at the same concentration. The result

of the corrosion study of mild steel has proved that succinyl amide gemini surfactant is an efficient inhibitor of mild steel corrosion in 0.5 M HCl.

Production of Pure Pinolenic Acid from Pine Nut Oil via Enzymatic Esterification Combined with Urea Complexation

D. No[(1)], T. Zhao[(2)], I. Kim[(3)]
[(1)]Korea University, Korea, Republic of [(2)]Korea University, Korea, Republic of [(3)]Korea University, Korea, Republic of

Pinolenic acid (PLA, C18:3?5) known as a beneficial and unique fatty acid is not available as a pure form. In the present study, pure PLA (>99% purity) was successfully produced from fatty acid from pine nut oil through two-step process including selective esterification using a lipase from Candida rugosa and urea complexation. For the first step, lipase-catalyzed esterification between fatty acid from pine nut oil and lauryl alcohol was carried out at the molar ratio of 1:1 (fatty acid to lauryl alcohol). Three parameters, namely enzyme loading, initial water content in the substrate and temperature were investigated to optimize the reaction condition. Optimum condition of enzyme loading, initial water content, and temperature were 0.1%, 10%, and 5 oC, respectively. At this condition, a maximum PLA content of 43% was obtained from starting material containing 13% PLA. For the second step, urea complexation using PLA-enriched fatty acid from the first step was conducted. As a result, a pure PLA (>99% purity) was obtained at the ratio greater than or equal to 1:4 (fatty acid to urea).

AFTERNOON

BIO 5: Biotechnological Advances for Oilseed Improvements

Chair(s): T. McKeon, USDA, ARS, WRRC, USA; R. Wilson, Oilseeds & Bioscience Consulting, USA

A Rapid Nile red Fluorescence-based Method for Quantification of Triacylglycerol in Cell Suspension Cultures of Brassica Napus

Y. Gao[(1)], R. Siloto[(2)], R. Weselake[(3)]
[(1)]University of Alberta, Canada [(2)]University of Alberta, Canada [(3)]University of Alberta, Canada

Microspore-derived cell suspension cultures of Brassica napus L. cv Jet Neuf are a useful experimental system for studying triacylglycerol (TAG) accumulation. Quantification of the TAG content of plant cells and tissues, however, can be time consuming when using traditional lipid extraction in combination with TLC to isolate TAG and GC quantify the fatty acid methyl esters derived from TAG. In the current study, the TAG content of cell suspension cultures was quantified by a Nile red fluorescence ?based assay using a Synergy H4 hybrid multi-mode reader. Cultures with different TAG content were generated by adjusting the concentration of carbon and nitrogen supplements in the growth medium. TAG was quantified based on an excitation wavelength of 490 nm and emission wavelength of 595 nm. The fluorescence was not influenced by other lipid classes or other cellular components. Fluorescence intensity increased linearly with the TAG content cells, as quantified by a more traditional method. The Nile red fluorescence-based method was used to demonstrate that the TAG content of the cell suspension cultures varied with the growth stage of the culture and could be used to monitor the TAG content of these cells in a high throughput fashion.

The renewed database "Seed Oil Fatty Acids" (SOFA)

B. Matthäus[(1)]
[(1)]Max Rubner-Institut, Germany

Since April 2012 the database ?Seed Oil Fatty Acids (SOFA)? is available again on the internet free of charge after registration (www.sofa.mri.bund.de). In cooperation with the company Comicon GmbH (Hamburg, Germany), specialized on programs with chemical background, and by financial support of the German Federal Ministry for Nutrition, Agriculture and Consumer Protection supplied by the Fachagentur Nachwachsende Rohstoffe e. V. a new database system was created (FKZ: 20014408). The database comprises 580 different fatty acid structures and more than 7,000 plant species. The information of about 130,000 individual percentage data from fatty acid tocopherol, phytosterol and triacylglycerol composition as well as some physical data of seed oils occurring in plant seeds is distributed on more than 18,000 tables. The information was collected for more than 40 years from appropriate pharmaceutical, botanical and chemical literature by the Max Rubner-Institut (MRI). The strongest feature of the database is the delta-notation allowing the search for fatty acid structures or partial structures such as *9a* (acetylene in position 9), or *5t,9c* (for 5-trans,9-cis). By this it is possible to find the occurrence, percentage level and distribution of such structures in the plant kingdom. The database may be very useful for chemists, biochemists and food scientists as well as for botanists to find information not only for renewable resources and "green" chemistry, but also for gene technology, for understanding the enzymes of fatty acid biosynthesis and their mutations during the evolution of plant families and species, for plant chemotaxonomy and for systematic and phylogenetic botany.

Chemoenzymatic Method for Producing Stearidonic Acid Concentrates From Stearidonic Acid Soybean Oil

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The aim of this study was to produce stearidonic acid (SDA, 18:4 n-3) omega-3 concentrates from 25% SDA soybean oil (SDASO) by enzymatic acidolysis. Substrates were prepared by chemical and enzymatic hydrolysis of SDASO. A 62% SDA free fatty acid fraction (SDA-FFA) was obtained by low temperature crystallization of the chemical hydrolyzate while selective hydrolysis of SDASO by C. rugosa yielded a 51% SDA acylglycerol mixture. Process conditions for acidolysis between SDA-FFA and the acylglycerol mixture were optimized using response surface methodology (RSM). Incorporation of SDA into acylglycerols by Lipozyme RM IM and C. cylindracea lipase (CCL) was mathematically modeled under varying levels of substrate molar ratio (Sr), incubation temperature (Temp) and time (t). Process conditions for production of a 60% SDA concentrate was predicted to be Sr = 4.8, Temp = 65 oC and t = 8h for Lipozyme RM IM and Sr = 5.0, Temp = 43 oC and t = 48h for CCL. The model was verified experimentally by gram scale synthesis under these conditions which resulted in production of 59.98 and 58.98% SDA concentrates (? 96% triacylglycerols), by Lipozyme RM IM and CCL, respectively. The SDA omega 3 concentrates produced may be used for formulating nutritionally functional foods.

Enrichment of Stearidonic Acid from Echium Oil via Two Step Lipase-Catalyzed Esterification

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Stearidonic acid (SDA) from echium oil was enriched substantially by a two step lipase-catalyzed esterification using Lipase OF from Candida rugosa and Lipozyme RM IM from Rhizomucor miehei in a solvent-free system. The first step was attempted to enrich the SDA via Candida rugosa lipase-catalyzed esterification of the fatty acids from echium

oil with lauryl alcohol. It was observed that SDA was enriched in the unesterified fatty acid fraction of reaction mixture. The effects of amount of water, temperature, and enzyme load were investigated. The optimal reaction condition was 0.25% for the amount of water, 30°C for temperature, and 1% for enzyme loading when both the content and the yield of SDA are considered. Under this condition, the maximal SDA content of 40% was obtained from the starting material containing 14% SDA. To further elevate the SDA content, Lipozyme RM IM-catalyzed ethanolysis using the SDA enriched fatty acid from the first step was carried out and the highest SDA content of 57% was obtained. Through these two step lipase-catalyzed esterification, the SDA content in echium oil increased fourfold from 14% to 53% with 85% of yield.

Development of Plant-derived Omega-3 Fatty Acids Through Biodiversity.

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The purpose of this presentation is to illustrate how development of new agricultural crops through biodiversity has potential to alleviate the world?s impending shortage of dietary omega-3 fatty acids. Long chain omega-3 fatty acids from marine sources possess proven health benefits which have resulted in increased demand for these materials. However, shrinking global supplies of marine omega-3s, coupled with the relatively poor bioavailability of existing plant derived omega-3 fatty acids, such as alpha linolenic acid (ALA) from crop sources like flax and chia, is now creating significant challenges for the food and dietary supplement industries. Crop production has the potential to help fulfill market demands for efficacious alternatives to marine sources of omega-3 fatty acids. Although some effort is being directed to achieve this through biotechnology and genetic modification of crops such as canola, it can also be achieved through biological diversity (biodiversity). Crop biodiversity is the natural variance in the genetic characteristics of plants used in agriculture and development of new crops through biodiversity has been practiced for centuries. It is possible to identify variation in almost every trait of a plant species, including nutritional qualities such as omega-3 content. Recent developments have resulted in AhiflowerTM, a new crop from Buglossoides arvensis that yields increased levels of omega-3 stearidonic acid (SDA), which is 4-6 times more bioavailable than ALA. This presentation will discuss the advantages of using biodiversity for new crop development, and provide an overview of the new crop development process, using AhiflowerTM as an example.

Physical & Chemical Properties of Salvia Hispanica Seeds in Comparison With Very High Fiber & ?3 Sources

Among the highest ?3 sources Salvia hispanica lines have as much as ~ 65% ?-linolenic acid and are among the highest dietary sources of healthful fiber. The fiber has enormous water adsorbing properties forming extensive hydrogels. S. hispanica seeds are actually nutlets or dried fruits. Seeds or nutlets with the highest known ?3 fatty acid levels are members of the Lamiaceae family. Nutlets of several other members of the Lamiaceae have long been considered to have considerable health value particularly Ocimum basilicum and Hyptis suaveolens. The hydrogel properties of S. hispanica, O. basilicum and H. suaveolens nutlets were compared to those of psyllium and flax seeds. These seeds/nutlets rapidly form hydrogels at many times the initial volume with O. basilicum having the fastest and highest hydrogel formation, flax the slowest and H. suaveolens the least. The hydrogels vary widely in microstructure and carbohydrate polymer composition with mixtures of pectin-like, hemicelluloses-like and cellulose molecules. The most prominent monomers are xylose, arabinose and galacturonic acid. Growing evidence indicates the most important property of soluble fiber is the viscosity. Psyllium is considered one of the best fiber sources but we find that the viscosities S. hispanica, O. basilicum and H. suaveolens hydrogels are very much higher than those of Psyllium or flax. This suggests that these nutlets may be the most healthful sources of fiber known and their very high water holding capacities have important implications for healthier food and can replace much of the starch and saturated fat in common foods.

Addressing Issues in Bringing Back the Castor Plant as a Domestic Crop

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The castor plant Ricinus communis L. is the source of castor oil, an important chemical feedstock. The castor oil contains up to 90% of the hydroxy fatty acid ricinoleate which is used in producing lubricants, plasticizers, polyurethanes and monomers for production of long-chain polyamides. The castor plant is one of the most productive annual oilseeds, yielding up to the equivalent of 15 barrels of oil per hectare. It can be grown with very limited agricultural inputs and still provide over half a ton of oil per hectare. As a non-food crop, it provides suitable replacements for chemical products currently derived from petroleum. However, the castor seed also contains the protein toxin ricin, creating a serious disposal issue for the seed meal remaining after oil extraction. Although castor was once grown and processed in the US, concern about the toxin has inhibited its re-introduction. We are addressing the toxin issue in a several ways. We have found that limited protease treatment in organic solvent, similar to an oil extraction process, will eliminate ricin protein. Microwave pretreatment of castor seed significantly also reduces ricin activity, although most of the ricin protein remains immunodetectable. We have also used ricin detection techniques to identify castor cultivars with lower ricin content for ultimate incorporation in a breeding program aimed at eliminating ricin from castor. Finally, we have identified approaches for reducing the impact of castor volunteers from contaminating major crops.

Biotechnology Poster Session

Chair(s): R. Ashby, USDA, ARS, ERRC, USA; J. Ogawa, Kyoto University, Japan

Maximizing Biodiesel Production From Yarrowia Lipolytica Po1g Biomass Using Subcritical Water Pretreatment

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The yeast Yarrowia lipolytica Po1g is one of the oleaginous microorganisms with a potential for biodiesel production. Sub-critical water (SCW) treatment has been known as an effective method for increasing the amount of extractable lipids in microorganisms. In this work, the amount of neutral lipids and fatty acid profiles in neutral lipids extracted from Y. lipolytica Po1g with and without SCW pre-treatment were investigated. The effects of temperature (125, 150 or 175 oC), amount of water (20, 30 or 40 mL/g bio-mass) and time (10, 20 or 30 min) showed that maximum neutral lipid (42.69%, w/w) could be achieved at 175 oC using 20 mL water for 20 min. The maximum neutral lipid from unpretreated samples was 23.21%. No difference in fatty acid profiles was observed, but long chain fatty acids were observed in higher amount in SCW pretreated samples. SCW pretreatment increased biodiesel yield twofold.

Subcritical Water and Dilute Acid Pretreatments for Bio-ethanol Production From Melaleuca Leucadendron Shedding Bark

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The feasibility of bio-ethanol production using the lignocellulose of the shedding bark of Melaleuca leucadendron (Paper bark tree) was investigated. The optimal pretreatment conditions were determined using a 4x3x3 factorial

design. At very low severity conditions (CSF?0.335), 28% of xylan was recovered and this recovery increased with increasing CSF till it peaked to 64.4% (11.2 g xylose L-1) at a CSF of 1.475. However, at CSF >2.0, xylose yield declined due to degradation. Mild and progressive glucose yield was detected in prehydrolysate at CSF?1.514, and subsequent enzymatic hydrolysis allowed complete glucan solubilization. Implementing environmentally friendly subcritical water pretreatment at CSF?0.335 on the shedding bark, about 85% of glucan solubilization was achieved after enzymatic hydrolysis. Saccharomyces cerevisiae readily fermented crude hydrolysate with in 12 h, yielding 24.7gL-1 ethanol at an inoculum size of 2% (v/v), representing a glucose to ethanol conversion rate of 0.475 g g-1 (93% fermentation efficiency). Based on our findings, the shedding bark is a potential feedstock for bio-ethanol production.

Rapeseed and Sunflower Meals: Efficient Substrates for Lignocellulolytic Enzyme Production by Filamentous Fungi

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Rapeseed and sunflower meal (RSM and SFM), the by-products of biodiesel production, are mainly composed of proteins, lignocellulosic fibres and minerals. Beside their use in animal feeding, RSM and SFM offer cheap, eco-friendly substrates rich in fibers, protein and energy contents suitable for the growth of microorganisms, especially filamentous fungi, opening new challenging perspectives in the production of enzymes and other bioproducts (antibiotics, antioxidants, vitamins, biogas and bio-oil). The aim of this study was one hand to evaluate the use of RSM and SFM as substrate for several filamentous fungi in solid-state fermentation (SSF) or as a supplement to the submerged production medium and, on the other hand, to determine the lignocellulolytic enzyme production.

Production and Microencapsulation of Structured Lipids Enriched With Sn-2 Palmitic Acid and Long-chain Polyunsaturated Fatty Acids for use in Infant Formula

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The differences in stereospecific structure of triacylglycerols (TAGs) in vegetable oils used in infant formula relative to those in breast milk fat lead to lower energy and calcium absorption by formula-fed infants. The aim of this study was to produce structured lipids (SLs) with a similar amount of palmitic acid esterified at the sn-2 position as present in breast milk fat. In addition, physiologically important fatty acids such as arachidonic (ARA), docosahexaenoic (DHA), and gamma-linolenic (GLA) acids were incorporated in the SLs glycerol backbones. These SLs were produced via acidolysis catalyzed by sn-1, 3 specific and non-specific lipases. The SLs contained approximately 50 % (w/w) palmitic acid at the sn-2 position and approximately 10, 5, and 5 % (w/w), ARA, DHA, and GLA, respectively. Formulation of infant formula using these SLs was developed. High level of unsaturation made these SLs prone to oxidation. Microencapsulation using Maillard reaction products as encapsulants was employed to deliver these SLs into infant formula system. The results from this study provided methods to produce human milk fat analogs with high level of fatty acids important in brain development and cognitive functions as well as methods to deliver them into infant formula system.

Kinetic Study and Modeling of Biosynthesis of the Flavor Precursors, Linoleic Acid Hydroperoxides, Using Commercial Soybean Lipoxygenase

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Among the polyunsaturated fatty acids (PUFAs), containing the E,E-1,4-pentadiene system, linoleic acid (LA) is considered as being the substrate model for the biosynthesis by lipoxygenase (LOX) of flavor precursors, linoleic acid hydroperoxides (HPODs). The aim of this research was to study the kinetic behavior of a commercial soybean LOX in the bioconversion of LA into HPODs and to propose a mathematical model for the prediction of the performance of the enzymatic system. The Km and Vmax values of 343.1 mM and 2.5 mM/min, respectively, for LOX, using a

commercial LA (67%), were calculated from Lineweaver-Burk plots of 1/v versus 1/[S], whereas the inhibition constant Ki of 105.9 mM was determined from the Dixon plots. Using the experimentally determined kinetic parameters along with the Michaelis-Menten equations, the bioconversion of LA into HPODs by LOX was simulated with MATLAB program. The MATLAB hence was used to predict the evolution of LA conversion and HPODs synthesis in function of time. The experimental results indicated that there was a correlation between the profile of the synthesized HPODs, determined experimentally, and that of the predicted one obtained by simulation. A model, based on Michaelis-Menten kinetics, that could describe the enzymatic oxidation of LA by soybean LOX, was successfully obtained.

Lipase-catalysed Enrichment of GLA From Evening Primrose oil in a Solvent-Free System

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?-Linolenic acid (GLA) was successfully enriched by enzyme-catalysed esterification of evening primrose oil fatty acid (EPO-FA) and 1-butanol (BtOH) in a solvent-free system using Lipase OF from Candida rugosa as a biocatalyst. The parameters evaluated were substrate molar ratio (1:4, 1:6, 1:18, 1:10 & 1:12, EPO-FA:BtOH), temperature (10, 20, 30, 40, 50 & 60oC) and enzyme loading (5, 10, 15 & 20%, with respect to substrates mixture). The effect of addition of molecular sieve at optimum condition was also investigated. Optimum conditions were a temperature of 30°C, an enzyme loading of 10%, and a molar ratio of 1:10 (EPO-FA to BtOH). A maximal GLA content of 83.7% was obtained when molecular sieve was applied under optimum condition.

Alternations in Biosynthesis and Production of Fungal Polyunsaturated Fatty Acids by Metals

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Polyunsaturated fatty acids (PUFAs) are one of the main focuses of nutritional-lipid industry. Because of inadequacy of natural PUFAs sources, biotechnological production of these biologically active compounds provides a new challenge in this field. Satisfied microbial producers of PUFA are Zygomycetes fungi, e,g, Thamnidium, Mucor, Cunninghamella, and Mortierella. These fungi are classified as ?oleaginous? microorganisms that accumulate high amount of lipids in cells with sufficient content of.PUFAs. However, high content of PUFAs in fungal oil depends on optimal cultivation conditions. Metals are one of the key factors that influence both lipid accumulation and fatty acid composition. This work deals with the study of various metals (Zn2+, Fe2+, Cu2+, Se4+) involvement to fatty acid biosynthesis by Thamnidium elegans (producers of gamma-linolenic acid - GLA). Individual metals were added to the media in exponential growth phase. It was found that Zn2+ and Fe2+ ions caused 3-5 times increased in stearic acid content compare with control. Nevertheless, different actions of these two metals on fatty acid desaturases were observed. While zinc inhibited delta-9 desaturase, iron predominantly reduced delta-12 desaturase activity. Oppositely, Cu2+ (up to 0.2 mM) stimulated both lipid biosynthesis and GLA yield (up to 1.5 times). Interestingly, Se4+ ions stimulated formation of n-3 biosynthetic pathway and content of both alpha-linolenic and stearidonic acid was doubled compare with control. Presentation describes participation of these metals in individual desaturation steps of n-6 and n-3 PUFA pathways. The work was supported by grant VEGA 1/0975/12 and by grants APVV-0662-11 and APVV-0294-11.

The Giant Panda and Biofuels: Metagenomics and Anaerobic Bacteriology

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The use of giant panda feces may hold the key to reducing the cost of biofuel production. Next-generation sequencing elucidated seventeen cellulolytic and seven oleaginous microorganisms that can be used in the production of

lignocellulosic biomass-based biofuels from the giant panda?s fecal metagenome, and the presence of several organisms have been validated using species-specific PCR (Clostridium cellulovorans, Streptococcus gallolyticus, Saccharophagus degradans, Thermanaerobacterium themosacchrolyticum, Shewanella piezoltolerans, Pseudomonas fluorenscens and P. putida). These microbes can be used to pretreat lignocellulosic biomass, converting the biomass into simple sugars that can be used by oleaginous microbes to accumulate lipids. These lipids can be converted into biodiesel; thus creating a usable product from two waste materials and lowering costs associated with biofuel production. In our preliminary study, these organisms have been shown to degrade cellobiose (cellulose surrogate) and accumulate transesterifiable lipids under anaerobic conditions. Analysis of sugar samples indicate that time and cellobiose concentration effects are significant with respect to sugar consumption (time, P=0.0015; concentration, P<0.0001). Analysis indicates that 65.4 % of cellobiose was consumed, and that transesterifiable lipids were accumulated. Further characterization of the microbes is currently underway to evaluate the activity of the enzyme systems and processes associated with these metabolic pathways.

Enzymatic modification of phosphatidylcholine with n-3 polyusaturated fatty acid using immobilized phospholipase A1

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Phosphatidylcholine (PC) was successfully modified by acidolysis of PC with n-3 polyunsaturated fatty acids (n-3 PUFA) obtained from fish oil using an immobilized phospholipase-A1 from Thermomyces lanuginosus/Fusarium oxysporum as a biocatalyst. Effect of several parameters such as water content, temperature, and enzyme loading were studied to determine the optimum conditions for the acidolysis reaction at the molar ratio of 1:8 (PC to fatty acid). Throughout all the reactions, an inverse relationship between incorporation of n-3 PUFA and PC recovery was observed. Optimum conditions of initial water content, temperature, and enzyme loading were 1%, 55?C, and 20%, respectively. Under these conditions, the highest incorporation (57 mol%) of n-3 PUFA into PC was obtained and the yield of PC was 16 mol% at 24 hr of reaction time. Vacuum system was applied after 3 and 9 hr of reaction time under atmospheric pressure to enhance the yield of PC. Under vacuum, incorporation of n-3 PUFA into PC decreased slightly, but yield of PC increased significantly as the reaction time increased.

Synthesis of structured triacylglycerols rich in n-3 PUFA by acidolysis of soybean oil using native lipases

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Structured triacilglycerols (STAGs) by incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into soybean oil for nutraceutical purposes catalyzed by native lipases (Aspergillus niger- AN and Rhizopus javanicus-RJ) were obtained by acidolysis of soybean oil with a free fatty acid mixture obtained from sardine oil (sardine-FA). First, different lipase/support ratios were tested for the immobilization of lipases, and the best results were obtained with ratios of 1:4 (w/w) for lipase AN and 1:7 (w/w) for RJ using Amberlite MB-1. Both lipases were stable for at least 3 days in the operational conditions and maintained constant activity for 2 months in the storage conditions (5?C). A fractional experimental design was carried out to study the effect of different parameters on STAG production for each lipase. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) was used for instantaneous characterization of STAGs. STAGs with 6.2 - 11.7 % of EPA + DHA were obtained, leading to a significant reduction in the n-6/n-3 FA ratio of soybean oil. The Aspergillus niger lipase was the most effective in concentrating n-3 PUFA. The best reaction conditions to achieve an adequate n-6/n-3 FA ratio were: initial water content of the enzyme of 0.8 % (w/w), sardine-FA:soybean oil mole ratio of 3:1, reaction time of 24 h, reaction temperature of 40?C and 7 % of lipase (w/w). After the enzymatic reaction, a great variety of new STAGs were formed containing EPA, DHA or both in the same molecule.

Effects of enzymatic interesterification on physicochemical properties of blends of palm stearin, palm kernel oil and olive oil to produced trans-free margarine analags.

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The consumer is becoming more aware of the relationship between diet and disease, which has driven the research on functional foods and their effects on the body. The role of fats and oils in human nutrition has been intensively studied and discussed for decades. It has been emphasized the importance of intake of omega-3, omega-6 and omega-9 fatty acids, reduction of saturated fatty acids and, more recently, control of intake of trans fatty acids. Through the blend and interesterification of oils and fats, trans-free fats can be produced. Fat blends, formulated by ternary blends of palm stearin, palm kernel oil and olive oil were modified by enzymatic interesterification. The effect of enzymatic interesterification process was determined by comparing the chemical and triacylglycerol composition and regiospecific distribution of fatty acids in triacylglycerols. The enzymatic interesterification allowed obtaining fats with various degrees of plasticity, increasing the possibilities for the commercial use of palm stearin palm kernel oil and olive oil.

Preparation of oligo(Ricinoleic Acid Derivatives via Lipase-Catalyzed Esterification as Lubricant Additives and Star Polymers for Drug Delivery

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We have employed biocatalysis using immobilized lipases under solvent-free reaction conditions to convert ricinoleic acid as a model hydroxyl fatty acid biorefinery derivative to produce derivatives of its oligomers. Covalent attachment of oligo(ricinoleic acid) to polyols containing primary hydroxyl groups such as pentaerythritol produces star polymers that possess low melting point temperatures and high viscosity indices, suggesting their used in lubrication. Recently, we have investigated approaches to produce similar star polymers that would be more effective as drug delivery vehicles, to allow for a greater density of oligo(ricinoleic) acyl chains extending outward from the central core, and to possess functional groups on the termini of the chains, which will enable conjugation of hydrophilic groups, such as poly(ethylene glycol) and its derivatives, thereby producing a unimolecular polymeric micelle. The main approach is to enzymatically attach 10-undecenoic acid to the termini, to incorporate a reactive terminal double bond into the resultant product. This poster will provide an overview of the different approaches used to conduct and monitor the progress of reaction, the latter of which was quite challenging.

High level production of docosahexaenoic acid (DHA)-rich triacylglycerol by a novel strain of thraustochytrid

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Thraustochytrids, marine heterokonts, are known to accumulate a large amount of docosahexaenoic acid (DHA) in the triacylglycerol (TAG) form, however, improvement of the productivity is required to decrease the production cost. In this study, we screened more than 1,000 strains of thraustochytrids isolated from coastal area of Okinawa Island in Japan and a strain OH4 that showed superior growth and carbon consumption in the medium containing glucose was isolated. However, the ratio of DHA in the accumulated TAG in OH4 was not so high (30%). As the result of the analysis of accumulated TAG in OH4, it was shown that DHA was predominantly esterified in the sn-2 position, and for further improvement of DHA productivitiy, esterification in the sn-1 or sn-3 position was required. Thus, we isolated mutants from strain OH4 by chemical mutagenesis, in which a strain having a mutaion in the factors involved in the acylation of TAG would contain.. Among the mutants, we found a mutant strain LTR23 which showed increased DHA proportion compared to the parent strain. Positional analysis of the TAG revealed that DHA was esterified not only sn-2 position but sn-1 or sn-3 position. When the strain LTR23 was cultivated on glucose in a 30-L fermenter, LTR23 grew at the biomass level of 134 g/L (dry cell weight) and accumulated 44 g/L of DHA with the DHA ratio of 52%. The mechanism of the positional specificity of DHA will also be discussed.

Metabolic engineering approaches to increase the oil content of seeds

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Current transgenic approaches to increase the oil content of seeds include successful attempts to increase metabolic 'pull' toward oil biosynthesis through the increased expression of genes controlling glycerolipid assembly, such as diacylglycerol acyltransferase. Equally successful have been attempts to increase metabolic 'push' by the overexpression of metabolic regulators that enhance the expression of genes encoding enzymes of glycolysis and fatty acid biosynthesis. Examples of these approaches to increase the oil content of soybean without reducing protein content will be described. Recent attempts to identify control points for carbon partitioning among oil, protein and carbohydrates in developing seeds will also be described.

A one-step enzymatic process for high-maltose syrup and trehalose production using corn as substrate

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Trehalose is a novel multi-functional disaccharide to be used as a sweetener and useful compound to help preserve biomaterials in the food, cosmetic, and pharmaceutical industries because of its the stable biological macromolecules ability. In this study, we planned to convert the low cost of feed corn starch into high cost of trehalose by using enzymatic method. After grinding the raw material, the feed corn starch content was measured as 64.36 ± 0.01%. For the liquefaction of starch, the ?-amylase was used as first biocatalyst, and then add the ?-amylase and pullulanase at 35 ? for 12 h to saccharified the hydrolysate into high maltose syrup. The weight percentage of maltose conversion rate was calculated as 81.07 ± 1.16 %. The trehalose conversion reaction of Picrophilus torridus trehalose synthase (PTTS) was proceeded under 35 ? for 12 h and the maximum conversion rate was 50.91 ± 0.01 %.

Lipase-catalyzed Production of Fatty Acid Methyl Esters from Olive Pomace Oil in a Packed Bed Reactor

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Biodiesel industry is looking for alternative feedstocks for biodiesel production. In this study, response surface methodology based on central composite rotatable design was employed to study the immobilized lipase-catalyzed conversion of olive pomace oil, a by-product of olive oil industry, to fatty acid methyl esters (FAME, biodiesel) in a packed bed reactor, and to optimize the reaction conditions: temperature (25-65 °C), reaction time (2-10 h), substrate mole ratio (methanol:olive pomace oil, 1.5-7.5) and substrate flow rate (0.5-16.5 mL/min). Moderate temperatures, and higher reaction times and higher substrate mole ratios increased the FAME content, whereas substrate flow rate did not have a significant effect on the conversion. The optimal reaction conditions generated from the predictive model for the maximum FAME content were 51 °C, 8 h, 6 substrate mole ratio and 5.43 mL/min substrate flow rate. The optimum predicted FAME content was 75.4%, while the actual FAME content was 74.1±1.8%. Utilization of olive pomace oil as an alternative feedstock for biodiesel production at the countries where olive oil is produced may add value to the olive oil industry and the development of the rural economy.

Enzymatic production of FAME biodiesel with soluble lipases

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Biodiesel is a viable alternative to fossil fuels, and biocatalysis is gaining interest as a greener process. We focus on converting oils to Fatty Acid Methyl Ester (FAME) using soluble lipases, which offer an advantage compared to immobilized enzymes by cost efficiency and ease of implementation. Firstly, we defined the range of interest for process parameters of a low catalyst loading system, intended for single use. Furthermore we systematically studied the effect and interaction between these parameters. Based on experimental data, a model was developed to evaluate the

optimal conditions within the defined operating space concerning: temperature, water content, initial methanol concentration and enzyme content. The identified optimum range was experimentally evaluated, and model findings were confirmed. Another barrier in lipase use in biodiesel production is the higher melting point (m.p.) of certain oils, which is not compatible with the temperature range where lipases are most active. To address this, here we explored a novel production strategy that accommodates the enzymatic requirements with the chemical limits of the substrates. The m.p. of the methyl ester product is lower than that of the starting material. Thus, we have incorporated a varying amount of the product to lower the m.p. of the starting material. Our case study is the reaction of Palm Fatty Acid Distillate (PFAD) to FAME. Conversion rates have been measured with varying temperatures, water concentration, and initial methanol content. The results of this investigation will presented and discussed in this poster.

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