2011 Annual Meeting Abstracts

Biotechnology

MONDAY

MORNING

ANA 1 / BIO 1: Lipidomics and Metabolic Analysis

Chair(s): W.C. Byrdwell, USDA, ARS, USA; and R. Weselake, University of Alberta, Canada

Plant Lipidomics to Identify the Roles of Lipids in Plant Stress Responses. R. Welti[1], H.S. Vu[1], M. Roth[1], P. Tamura[1], S. Shiva[1], S. Sarowar[2], V. Nalam[2], G. Klossner[2], K. Lorenc Kukula[2], M. Li[3,4], G. Gadbury[1], J. Shah[2], X. Wang[3,4], [1]Kansas State University, Manhattan, KS, USA, [2]University of North Texas, Denton, TX, USA, [3]University of Missouri at St. Louis, St. Louis, MO, USA, [4]Danforth Plant Science Center, St. Louis, MO, USA

Our group is investigating how membrane lipids change when plants are exposed to environmental stresses. Direct infusion electrospray ionization triple quadrupole mass spectrometry, quadrupole time-of-flight, collision induced dissociation time-of-flight, and Fourier transform ion cyclotron resonance mass spectrometry are being employed to profile lipids as a function of plant genotype and treatment. Currently we are analyzing a large-scale experiment examining the role of oxidized lipids in plant response to biotic and abiotic stresses, including freezing, salinity, bacterial infection, and fungal infection.

Acylated Monogalactosyldiacylglycerols: Their Detection and Possible Biological Roles in Plant Stress Responses. H.S. Vu[1], R. Welti[1], M. Roth[1], P. Tamura[1], S. Shiva[1], S. Sarowar[2], V. Nalam[2], M. Li[3,4], G. Gadbury[1], J. Shah[2], X. Wang[3,4], [1]Kansas State University, Manhattan, KS, USA, [2]University of North Texas, Denton, TX, USA, [3]University of Missouri at St. Louis, St. Louis, MO, USA, [4]Danforth Plant Science Center, St. Louis, MO, USA

Although acylated monogalactosyldiacylglycerols were first discovered almost four decades ago, they have been ignored due to the lack of evidence to support their biological roles. We have developed various mass-spectrometry-based methods to profile monogalactosyldiacylglycerols acylated with both oxidized and non-oxidized fatty acids. Many acylated monogalactosyldiacylglycerols significantly increase in Arabidopsis leaves challenged by different stresses. In responses to biotic stresses, oxidized acylated monogalactosyldiacylglycerols are induced to a much greater extent by avirulent bacterial interactions than by virulent interactions. When challenged by abiotic stresses such as mechanical wounding, freezing or salinity, unique patterns of induction are also observed.

Carbon Flux Analysis in Oil Crops. I.A. Guschina[1], M. Tang[1], U.S. Ramli[2], J.J. Salas[3], P.A. Quant[4], R.J. Weselake[5], J.L. Harwood[1], [1]Cardiff University, Cardiff, Wales, UK, [2]Malaysian Palm Oil Board, Kuala Lumpur, Malaysia, [3]CSIC, Seville, Spain, [4]Oxford University, Oxford, UK, [5]University of Alberta, Edmonton, Canada

Oil-producing plants represent an extremely important group of agricultural crops. The demand for their products has been increased recently by their use (and potential use) for chemical feedstocks, speciality nutraceuticals and, possibly, for biofuels. This is in addition to providing basic edible oils. In order to optimise oil production by crops we need to know how it is regulated both qualitatively and quantitatively. For the latter, flux analysis provides much useful information. We have applied one particular method (top-down flux control analysis) to a variety of important oil crops. Our data show that different crops show distinct properties, making wide generalisations difficult. However, it is possible to use the information from flux control analyses to inform genetic manipulations. This emphasises the importance, as well as the utility, of defining the details of carbon flux to plant storage oils.

Core Aldehydes of PtdCho as Possible Activators of Hydrolysis of Plasma Lipoproteins by Group IIA sPLA2. A. Kuksis, A. Ravandi, W. Pruzanski, University of Toronto, Toronto, ON, Canada

Recent studies have demonstrated that bee venom PLA2 (group III PLA2), which possesses structural similarity to group IIA sPLA2, is activated in liposomes by 1-palmitoyl-2-(9?-oxo-nonanoyl) GroPCho (a core aldehyde) and has suggested that other lipid oxidation products might produce similar effects. Other work had shown that group IIA sPLA2 preferentially attacked the hydroxyl and hydroperoxy linoleates and other oxygenated fatty acids, which were released from PtdCho of plasma lipoproteins at early times of incubation. Later oxygenated arachidonates (isoprostanes) were also identified among the products of plasma lipoprotein hydrolysis by group IIA sPLA2. In the present work we have identified both 1-palmitoyl(stearoyl)-2-[(5-oxo- valeroyl(9-oxo-nonanoyl)] GroPCho among the products of group IIA sPLA2 incubation of HDL and LDL of normal and acute phase plasma. The aldehydes were identified by LC/ESI-MS with reference to synthetic core aldehyde standards. The above liposomal studies and the present demonstration of formation of core aldehydes during a prolonged enzyme digestion provides a plausible explanation for the increased activity of group IIA sPLA2 observed occasionally with PtdCho, but which until now had remained unaccounted for.

Rapid Characterization of Lipids by MALDI MS. J.O. Lay, Jr., J. Gidden, R Liyanage, University of Arkansas, Fayetteville, AK, USA

Lipids can be analyzed after minimal processing in crude extracts without resort to hydrolysis and esterification. For glycerol lipids direct MALDI results in detection of sodium adduct ions of intact triacylglycerols (TAGs) and abundant diacylglycerol (DAG) like fragments. Unless protonated molecule formation is suppressed these fragments are abundant and preclude detection of DAGs in mixtures. DAGs like fragments can be eliminated by addition of base to preclude formation of unstable precursor protonated molecules during ion formation. With phospholipids the loss of the head group can be competitive with fatty acid loss. This can be useful for elucidation of phospholipids class. Indeed direct MS/MS of phospholipids in crude mixtures is very useful for the confirmation of the lipid class when obtaining phospholipids profiles because of the ability to establish parent-ion and product-ion relationships directly from mixtures. Lipid mixtures containing both glycerol- and phospho-lipids show only the

phospholipids by direct MALDI because of suppression of the TAGs by the more polar phospholipids. We have developed rapid SPE separation approaches to produce separate TAGs and phospholipids fractions. We have applied these techniques to the analysis of lipids, lipids-decomposition, olive oil adulteration, microbial taxonomy, lipid metabolism/biochemistry, and lipid oxidation.

Triple Parallel Mass Spectrometry (LC1/MS3) Method for Lipidomic Analysis of Vitamin D and Plant Triacylglycerols in Dietary Supplement Capsules. W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, Beltsville, MD, USA

Three methods are demonstrated for a complete analysis of vitamin D and triacylglycerols (TAGs) in fortified dietary supplements that virtually eliminates all chemical pretreatment prior to analysis. Three mass spectrometers, in parallel, plus a UV detector, an evaporative light scattering detector (ELSD), and a corona charged aerosol detector (CAD) were used simultaneously. The contents of gelcaps that contained 1000 IU (25 mcg) vitamin D3 in safflower oil and 2000 IU (50 mcg) vitamin D3 in rice bran oil were analyzed without the need for lengthy saponification and extraction. Three to five gelcaps were analyzed, each in triplicate or quintuplicate, using vitamin D2 as an internal standard. Vitamin D3 was analyzed using UV detection, selected ion monitoring (SIM) atmospheric pressure chemical ionization mass spectrometry (APCI-MS), and two transitions of multiple reaction monitoring (MRM) APCI-MS. The triacylglycerols in the oils were analyzed using full-scan APCI-MS, electrospray ionization (ESI) MS, up to MS4, the ELSD and the CAD. Triacylglycerols (TAG) containing fatty acids up to 28 carbons in length were identified. The gelcaps contained more than the label amount of vitamin D3 and differences were seen between synthetic vitamin D and that from fish oil.

LC-MS/MS as a Tool for Probing Industrial Oil Biosynthesis in Seeds. J.M. Dyer[1], T.R. Larson[2], L. Whitehead[2], A. Gilday[2], C.R. Dietrich[3], P. Yang[3], J.M. Shockey[4], C. Lu[5], E.B. Cahoon[6], I.A. Graham[2], [1]USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ, USA, [2]Center for Novel Agricultural Products, University of York, York, UK , [3]Donald Danforth Plant Science Center, Saint Louis, MO, USA, [4]USDA, ARS, Southern Regional Research Center, New Orleans, LA, USA, [5]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT, USA, [6]Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA

The seed oils of crop plants, composed primarily of triacylglycerols (TAGs), represent a major source of calories for human and animal nutrition and an excellent feedstock for the production of biofuels. In recent years, there has been increasing interest in engineering plants to accumulate high amounts of industrially important fatty acids in the oil, which can substitute for similar types of oleochemicals that are typically derived from non-renewable petroleum. The genes and enzymes required for producing high amounts of industrial oils in plants, however, are not fully understood, and host plants often respond in unexpected ways that limit the production of desired fatty acids. As such, sophisticated analytical techniques are needed to help identify the enzymes and metabolic processes that influence the production of industrial fatty acids in seeds. We have recently developed LC-MS/MS procedures for identifying and quantifying individual TAG molecular species. Application of these techniques to the study of plants producing either

hydroxylated or conjugated fatty acids has shed light on both the mechanisms of fatty acid "channeling" into TAG, as well as several bottlenecks that limit the production of industrial fatty acids in developing seeds. Implications for producing industrial oils in transgenic plants will be discussed.

Applying Genomics and Biotechnology to Design Soybeans for 21st Century Markets. Richard F. Wilson, United Soybean Board, Raleigh, NC, USA

Future gains in U.S. soybean productivity will be a function of superior technology. Advances in soybean genetics and biotechnology will: 1) help ensure an adequate supply, 2) provide cost-effective ways to adapt to government regulations, 3) help sustain domestic livestock markets, and 4) provide health conscience consumers with high quality foods. The United Soybean Board has enabled genetic modifications of soybean composition and yield enhancement that are now filling seed-industry pipelines with a series of elite soybean cultivars distinguished by specific quality traits. For example, soybeans that produce high-oleic oils under private labels are now becoming commercially available. Looking forward, knowledge gained from the sequence of the soybean genome will accelerate the development and deployment of additional traits that will continue to reset the ingredient paradigm for food and feed products. Soybeans with high levels of omega-3 fatty acids, and soybean meal with greater amounts of digestible phosphorus are among the soybean quality trait innovations. This review envisions how these next generation technologies will influence soybean consumption and trade in oilseed markets that are increasingly driven by customer demand for quality, nutrition and value.

Sterol Glycosides in Various Plant Materials Reflect Unique Sterol Patterns. L. Nyström[1], A. Schär[1], A.-M. Lampi[2], [1]ETH Zurich, Zurich, Switzerland, [2]University of Helsinki, Helsinki, Finland

Plant sterols from various plant materials have gained significant scientific interest in the past decades due to their use in functional foods. Recent studies have shown that also glycosylated sterols and natural intake levels of plant sterols can inhibit the cholesterol absorption in the gut. Very little information is available of the occurrence of these polar sterol conjugates in food materials, and even less information is available on the sterol composition found as sterol glycosides. We have demonstrated that the sterol composition as sterol glycosides (SG) and acylated sterol glycosides (ASG) can be significantly different from the total sterol composition found in various edible plant materials (e.g. beans, seeds, fruits, vegetables), and that there are unique patterns of sterol composition in SG from various plant families. Unlike commonly stated in the literature, sitosterol may not always be the most abundant sterol in the glycosylated sterols. Due to their more polar nature, incorporation of glycosylated sterols provides new dimensions to formulation of plant sterols in functional foods. Further, the sterol composition of SG mixtures may be modified by selection of SG source.

Biotechnological Approaches to Remove Chlorophyll Components in Plant Oils. Rene Mikkelsen[1], Janne Brunstedt[1], Birgitte Wittschieben[1], Heidi Pedersen[1], Lis Byrsting Møller[1], Charlotte Poulsen[1], Masoud Zargahi[1], Susan Madrid[2], Ken Carlson[3], [1]Danisco, Brabrand, Denmark, [2]Danisco USA, Palo Alto, CA, USA, [3]Danisco USA, New Century, KS, USA

Plant oils contain a vast number of compounds which potentially interfere with processing, stability, organoleptic properties, health effects etc of the final oil. For example, vegetable oils derived from oilseeds such as soybean and rape seed (canola) typically contain some chlorophyll which has to be removed in the final oil. Modern biotechnology provides a new powerful toolbox to modulate and control the properties of various components found in plant oils. Part of this toolbox is recombinant DNA and protein engineering technologies that make it is possible to create new enzymes targeted specifically towards selected compounds found in plant oils. This combined with modern and improved enzyme production capabilities have made it possible to generate new enzyme solutions for the fats and oil industry. We have isolated enzyme candidates from the chlorophyllase family of enzymes and tested the ability to degrade different chlorophyll components in an oil matrix.

BIO 1.1: Biocatalysis I

Chair(s): C.T. Hou, USDA, ARS, NCAUR, USA; and S.H. Yoon, KFRI, Korea

Enzymatic Synthesis of Chiral Intermediates for Development of Drugs. Ramesh N. Patel[1,2], Sandip J. Parekh[2], [1]SLRP Associates, LLC, Bridgewater, NJ, USA, [2]Unimark Remedies, Ltd., Mumbai, India

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

Protein Engineering of Lipases to Alter Fatty Acid Selectivity. Uwe Bornscheuer, Henrike Brundiek, Andrew Evitt, Robert Kourist, Institute of Biochemistry, Greifswald University, Greifswald, Germany

In this lecture, examples will be given for the protein engineering of lipases by rational protein design and focused directed evolution. This resulted in mutants with altered selectivity towards fatty acids.

Potential Metabolisms of Lactic Acid Bacteria for Functional Food Production and Probiotics. Jun Ogawa[1], Shigenobu Kishino[1], Akinori Ando[2], Kenzo Yokozeki[1], Sakayu Shimizu[1,3], [1]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan, [2]Research Division of Microbial Sciences, Kyoto

University, Sakyo-ku, Kyoto, Japan, [3]Faculty of Bio-environmental Science, Kyoto Gakuen University, Kameoka, Kyoto, Japan

Based on analysis of lipid and nucleic acid metabolisms, we proposed novel applications of lactic acid bacteria for lipid modifications and anti-hyperuricemia probiotics. 1) In order to develop flavor molecules of fermented food such as cheese, it is important to control the release of short-chain fatty acid from lipids. Tributyrin-hydrolyzing activity was screened in lactic acid bacteria, and Enterococcus faecium was selected as a potential strain. The tributyrin-hydrolyzing enzyme was purified, characterized and the gene was cloned and expressed in Escherichia coli. The enzyme showed specific hydrolyzing activity for triacylglycerol with short chain fatty acid. 2) Conjugated fatty acids have attracted much attention as a novel type of biologically beneficial functional lipid. Some lactic acid bacteria were found to be good catalysts for conjugated fatty acid production. 3) Hyperuricemia is a disease, which results from the over accumulation of uric acid and is greatly influenced by a high dietary intake of purine. Lactic acid bacteria were screened for their ability to degrade nucleic acid metabolites, and potential strains were selected. Their effects on the serum uric acid level were investigated using rats, and the ability to lower elevated level of serum uric acid was found in Lactobacillus strains.

Physiological Activities of Hydroxyl Fatty Acids. Ching T. Hou[1], Souren Paul[2], Sun C. Kang[2], [1]Renewable Product Technology Research Unit, NCAUR, ARS, U.S. D. A., Peoria, IL., USA, [2]Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Korea

In the search of value-added products from surplus soybean oil, we produced many new hydroxy fatty acids through microbial bioconversion. Hydroxy fatty acids are used in a wide range of industrial products, such as resins, waxes, nylons plastics, lubricants, cosmetics, and additives in coatings and paintings. Hydroxy fatty acids are also found to have antimicrobial and other physiological activities. In our continuous effort to screen natural products for their anti-microbial and enzyme inhibitor activities, we found that newly discovered trihydroxy fatty acids showed anti-plant pathogenic fungal activities and dihydroxy fatty acids had anti-bacterial activities. We also found that 10-Hydroxy-8(E)-Octadecenoic acid (HOD) exhibited strong anti-α-glucosidase (EC 3.2.1.20) activity. HOD is an intermediate in the bioconversion of oleic acid to 7,10-dihydroxy-8(E)-Octadecenoic acid (DOD) by a bacterial isolate, Pseudomonas aeruginosa (PR3). The α-glucosidase inhibitory activity of HOD was six times more potent than the commercially available anti-diabetic remedy, acarbose. Now, we are trying to screen microbial cultures from soil and water samples to find a better culture which is able to convert soybean oil directly to HOD to reduce the production cost of hydroxyl fatty acids.

Production and Modification of Functional Phospholipids Using Enzyme Reaction System. S.H. Yoon, Korea Food Research Institute, Seongnam-si, Kyunggi-do, Korea

All of the PLD tested, which are originated from Streptomyces chromofuscus, Streptomyces sp., Streptomyces prunicolor and Cabbage, are acceptable for the enzymatic transformation of phosphatidylcholine(PC) to phosphatidylserine(PS). And it was shown that the conversion of PC to PS was over 80% by using Streptomyces chromofuscus PLD. The maximum conversion was shown at the concentration of 25mM PC. And it is anticipated that higher conversion can be achieved even at the high concentration of PC, if it is possible to remove the liberated choline

from the reaction medium. The content of phosphatidic acid(PAc) that is a intermediate of transphosphatidylation was reduced around 5% by adoption of solid-liquid non-aqueous system instead of two-phase system where the content of PAc was from 10% to 20%, but the conversion of PC to PS was not changed. Though the conversion was not improved too much, the down steam process for the purification of PS can be simplified owing to the reduced Pac content. As the reaction medium for transphosphatidylation, ether and ester classes are good for the enzyme activity. However, in the case of nonionic polar solvents, no enzyme activity was detected.

Development of Biomaterials and Biofuel from Oilseeds. T. McKeon, USDA, ARS, WRRC, Albany, CA USA

Commodity oils are generally used for food or feed. Most of these oils contain a characteristic proportion of the 5 common fatty acids. Oils such as soy, canola are also used to produce a variety of oleochemicals. However, there are plants that produce seed oils containing fatty acids with unusual chemical functionalities. It is the chemical functionality of a vegetable oil that affects its uses by industry; chemical functionality can alter physical and chemical properties. One example is castor oil, containing 90% ricinoleate (12-hydroxy-oleate), which serves as a feedstock for lubricants, greases, sebacic acid and undecylenic acid. Tung oil, containing up to 80% of the conjugated fatty acid eleostearate is used directly in low volatile organic carbon (VOC) paints and architectural coatings. While these oils are commodity feedstocks and have been used for many years, there are some problems associated with producing them. Moreover, other fatty acids with high potential for use as chemical feedstocks, e.g. epoxy fatty acids, are produced in plants that have so far proven unsuitable as crops. There is considerable potential for development of oilseed crops that can provide biomaterials and feedstocks to the chemical industry and will expand the scope of applications for seed oils.

Recombinant Fusion Poly(hydroxyalkanoate) Synthase for Production of Biodegradable Polymer. D.K.Y. Solaiman, R.D. Ashby, Y. Liu, J.A. Zerkowski, USDA, ARS, ERRC, Wyndmoor, PA, USA

Poly(hydroxyalkanoates) (PHAs) are biopolymers synthesized by bacteria. PHAs are biodegradable and producible in industrial fermentation using renewable feedstocks. As a result, research abounds to develop PHAs into ′green′ substitutes for petroleum oil-based polymers, and limited commercial production of PHAs has commenced in several locations worldwide. We are interested in further improving product yield and modifying PHA composition using a genetic engineering approach. In this presentation, we report our study to construct and express fusion PHA synthase enzyme in recombinant bacteria in an attempt to increase PHA yield and modify its composition. We constructed an in-frame fusion gene (i.e., *phaEC*) composed of *phaE* and *phaC* genes of *Allochromatium vinosum* ATCC 35206, and subcloned it in an expression vector pBS29-P2-*gfp* to yield a recombinant plasmid, pBS29-P2-*phaEC*. This plasmid was introduced into *Ralstonia eutropha* and several *Pseudomonas* species by electroporation. Gene expression, PHA yield and PHA composition were determined using real-time-qPCR, gravimetric and GC/MS methods, respectively. The varying results obtained with the different bacterial hosts will be discussed.

Bioconversion of Marine Carotenoids and their Health Functions. Masashi Hosokawa[1],

Ching T. Hou[2], Kazuo Miyashita[1], Mi-Jin Yim[1], [1]Hokkaido University, Hakodate, Hokkaido, Japan, [2]NCAUR, ARS, USDA, USA

Fucoxanthin (FX), found in edible brown seaweeds, exhibits anti-obesity and anti-diabetic effects. In the body, FX is converted to fucoxanthinol (FXOH) and amarouciaxanthin A and accumulates in adipose tissue and other tissues. Therefore, it is suggested that FXOH and amarouciaxanthin A are active metabolites to show anti-obesity and anti-diabetic effects by FX. In addition, FXOH has been reported to have apoptosis-inducing effect on cancer cells and anti-oxidant activity. In this study, we, at first, examined the preparation of FXOH from FX by lipase-catalyzed hydrolysis. Porcine pancreatic lipase was an effective enzyme for FX hydrolysis. Taurocholate content in reaction system was important for reaction yield of FXOH. In our reaction system, FX was converted to FXOH at more than 85%. On the other hand, we also attempted FXOH preparation from FX through bioconversion with *Bacillus megaterium* ALA2. After 24 h incubation with strain ALA2 in culture media containing FX, we detected FXOH. This bioconversion is simple method to prepare FXOH.

Purification and Characterization of a Secondary Alcohol Dehydrogenase from Microalgae *Prototheca zopfii*. Eiji Sakuradani, Koji Kobayashi, Kei Nagao, Jun Ogawa, Kyoto University, Kyoto, Japan

The achlorophyllous microalgal genus *Prototheca* was found to have high activities of hydrocarbon-degradation in 1960's. Especially, *Prototheca zopfii* was proved to have high degradation activity of crude-oil hydrocarbons as well as *n*-alkanes. Aliphatic hydrocarbons are assimilated by various microorganisms such as bacteria, yeasts, fungi, and algae. In general, two major degradation pathways of acyclic hydrocarbons are known. The terminal oxidation pathway is widely found, whereas the sub-terminal oxidation pathway is just reported in limited microorganisms. Thus far, the genes and their enzymes related with the terminal oxidation have been studied in detail. However, the researches related with the microbial sub-terminal oxidation hardly progress. We found that *P. zopfii* oxidizes 5th carbon of *n*-hexadecane through the sub-terminal oxidation to generate 5-hexadecanol and 5-hexadecanone. We have purified a secondary alcohol dehydrogenase from *P. zopfii* through ammonium sulfate precipitation and six steps of column chromatography. Although the purified enzyme converted 2-dodecanol and 4-decanol to the corresponding ketones (2-dodecanone and 4-decanone), it never did a primary alcohol (1-dodecanol) to the corresponding oxidized compound.

AFTERNOON

BIO 2: Oil-based Biofuels

Chair(s): H.C. Holm, Novozymes A/S, Denmark; and M.J. Haas, USDA, ARS, ERRC, USA

Industrial Production of Biodiesel with Immobilized Lipases. S. Basheer, TransBiodiesel Ltd., Shfar-am, Israel

During the last two decades the biodiesel production process via lipase-catalyzed

transesterification/esterification reactions using plant oils and animal fats with short-chain alcohols has been extensively studied. Because of inhibition effect of short-chain alcohols, and subsequently the short operational life time of lipases and their high prices the production of biodiesel at industrial scales with a cost-effective enzymatic process remains unresolved issue. TransBiodiesel has developed new modified-immobilized lipase preparations capable of tolerating high concentrations of short-chain alcohols for the transesterification reaction of oils and fats with either methanol or other short-chain alcohols. Different oil feedstocks of low-grades have been tested including yellow and brown greases, animal fat and other acid oils. This presentation will cover the use of newly developed lipase preparations for the transesterification of animal fat and short-chain alcohols for the production of fatty acid alkyl esters in a lab-scale unit as well as in a semi-pilot demonstration system of 1500 litters in volume.

Important Details in Large Scale Enzymatic Catalyzed Biodiesel Production. P.M. Nielsen, M.L. Damstrup, A.R. Madsen, J. Brask, H.C. Holm, Novozymes A/S, Bagsvaerd, Denmark

The development of enzymatic catalyzed biodiesel production is entering into large scale production. The enzymatic process differs from the chemical catalyzed process in several important aspects. Firstly, enzymes works with lower surplus of alcohol in the reaction and is able to use ethanol instead of methanol with the positive impact on Green House Gas (GHG) reductions plus 5-6% more fuel from the oil raw material. Secondly, the enzymes can process free fatty acids as well as triglycerides which eliminate limitations to the content of free fatty acids in the raw materials and allowing low grade oils & fats for the process. On top of this we have succeeded in reducing the enzyme cost significantly by improving the efficiency in the lipase production and immobilization. The most important down side of the enzymatic process is the fact that it requires longer processing time in the transesterification which to some extent is compensated by less need for downstream processing/purifying of the biodiesel and glycerol. The different aspects of the enzymatic production will be discussed and illustrated by data to document the benefit of large scale enzymatic catalyzed biodiesel.

Enabling High Yield Biodiesel Production. P.M. Nielsen, Novozymes A/S, Denmark

Soluble Lipase-catalyzed Ethanolysis for Biodiesel Preparation. D. Liu, H. Ren, W. Du, Y. Sun, Dept. of Chemical Engineering, Tsinghua University, Beijing, China

Compared to immobilized lipase, soluble lipase-mediated biodiesel production has many advantages such as lower cost of enzyme and faster reaction rate. Our previous study showed that soluble lipase could effectively catalyze the methanolysis of renewable oil for biodiesel production. Ethanol, derived from renewable biomass, has a greater potential for biodiesel production in the future. We explored the potential of using ethanol as the acyl acceptor for soluble lipase NS81006-mediated biodiesel preparation for the first time in this paper. The effect of stirring rate, water content, molar ratio of ethanol to oil and ethanol adding strategy on soluble lipase NS81006-catalyzed ethanolysis was investigated systematically and an ethyl ester yield of 90% could be obtained under the optimized conditions. The reuse strategy of soluble lipase was further developed with a negligible loss in enzyme activity even after 10 batches running. The

results demonstrate that soluble lipase-mediated ethanolysis has great prospect in the field of biodiesel production.

Enzymatic Biodiesel Production: Evaluation of a Pilot Scale Operation. R. Burton, G. Austic, X. Fan, Piedmont Biofuels Industrial, Pittsboro, NC, USA

The search for alternative catalysts for the production of biodiesel has been of significant interest to industry.One primary reason to replace conventional alkaline catalysis is the elimination of soap contamination in commercial production. Furthermore, utilizing enzymes in the processing of fatty acid esters can reduce waste water streams, enhance the co-product quality of glycerol, and provide the ability to use lower quality feedstocks. These low quality feedstocks like yellow grease and brown grease with higher free fatty acid (FFA) content are largely underutilized for biodiesel due to the difficulty of processing these types of oils. This paper will evaluate the real world experiences of a pilot scale enzymatic biodiesel plant. This pilot scale operation has developed biodiesel production techniques using the immobilized enzymes Candida Antarctica Lipase B (CALB) and Thermomyces Lanuginosus TL-IM for the production of fatty acid esters..

Enzymatic Biodiesel, Analysis of the Reaction Kinetics. S.N. Fedosov, X. Xu, Agrobiology Group, Dept. Molecular Biology, Århus University, Århus, Denmark

Enzymatic production of biodiesel is characterized by a complex kinetic mechanism. It includes the following reactants: triglycerides (T), diglycerides (D), monoglycerides (M), fatty acids (F), alcohol (C), water (W), glycerol (G) and biodiesel (B). Multiple patterns of inhibition and inactivation complicate the situation. Previous attempts to analyze the whole process were somewhat ambiguous because a large number of parameters was applied to a limited number of points. In the present work, several independent partial reactions were examined using a minimal number of kinetic steps. A fluorescent signal was correlated to the concentrations of interest, which allowed multiple measurements and extrapolations. It was found that the enzyme follows the unsaturated kinetics, making possible a further reduction of coefficients, e.g. ks ≈ kcat / Ks. The below reaction patterns allowed a stepwise building of the scheme, e.g. B+W ↔ F+C (4 coefficients); B+G ↔ M+C (+1 coefficient); M+M+B ↔ G+C+D (+3 coefficients) etc. Additional patterns were discovered and added to the scheme in the course of work. The final model demonstrated a good predictability when simulating the reaction behavior of arbitrary oil mixtures.

Evaluation of FT-IR and FT-NIR Spectroscopies and Multivariate Calibration Models to Monitor Transesterification Reactions Progress. G. Güzel, X. Xu, Molecular Biology Instituite & Aarhus School of Engineering, Aarhus University, Aarhus, Denmark

Spectroscopic methods allow fast and non-destructive analysis of multi-component reaction systems without requiring complex pre-treatments (as in the case of chromatographic methods (GC)). Infrared and near-infrared spectroscopy (FT-IR/NIR) has recently become industrially popular and reliable methods for qualitative and quantitative analysis. Indeed, it is reported on the literature that the oils (feedstocks) and biodiesel (product) have similar mid- and near-infrared spectra. However, qualitative analysis of spectra by using statistical data processing methods, such as principal component analysis (PCA), reveals the spectral differences and thus

improves the correlations between spectral and analytical data. Accordingly, partial least squares (PLS) and principal component (PCR) regressions of such spectral data can be used to develop multivariate calibration models. This study evaluates application of FT-IR/NIR spectroscopy, statistical data processing methods, and thus developed calibration models that can be used to predict the analytical data from their spectra for transesterification reaction progress monitoring and determination purposes. Since such methods are less sensitive than chromatographic ones, results were also successively correlated with GC measurements.

TUESDAY

AFTERNOON

BIO 3 / H&N 3.1: Functional Lipids - Bioactive Properties

Chair(s): R. Moreau, USDA, ARS, ERRC, USA; and R.J. Ostlund, Washington University in St. Louis, USA

An Overview Of Functional Lipids. R.A. Moreau, ERRC, ARS, USDA, Wyndmoor, PA, USA

The main functions of lipids in plants,animals, and microbes are: a) to serve as a a source of energy and carbon skeletons and b) to serve as structural components in biological membranes. Although there is no authoritative definition of functional lipids, one can informally define them as a subset of functional foods, which are considered to be similar in appearance to conventional foods consumed as part of a usual diet, but they have been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. This symposium includes presentations from experts on seven functional lipids: xanthophylls, medium chain triglycerides, phytosterols, structured lipids, diacylglycerol oils, EPA-and DHA-rich oils, and tocotrienols. Each talk will include descriptions of the occurrence and chemical properties of each functional lipid and historical and recent physiological and clinical studies that provide evidence of the unique health-promoting properties of each of these seven functional lipids.

Medium Chain Triglycerides. P.J.H. Jones, Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada

Medium-chain triglycerides (MCTs) are dietary components that have been extensively examined for their role as weight control agents. MCTs, possessing fatty acids of 8-10 carbons in length, possess less energy per gram and are highly ketogenic compared to long chain triglycerides (LCTs). More importantly, however, MCTs compared to LCTs undergo distinct digestive and metabolic processes that may result in enhanced catabolism. Specifically, MCTs are rapidly oxidized and can be swiftly converted to energy, with less tendency to undergo deposition as body fat. Studies in animals and humans demonstrate that consumption of MCTs in low to moderate quantities may have substantial enhancing actions on thermic effect of food (TEF). For instance, sizeable differences in 24-h EE (energy expenditure) after consumption of 30 g of MCTs versus 30 g of LCTs have been shown; with these increases in EE theoretically resulting in about 0.5 kg of fat loss over a month if effects were to persist over that period.

However, even smaller doses of MCTs have been shown to cause larger diet-induced thermogenesis than LCTs. MCTs appear to increase EE as short chain FA are transported from gut directly to liver where they are catabolized immediately for energy which would be beneficial for weight loss. MCTs may also reduce body weight by enhancing satiety. These nutritional properties of MCTs are believed to upregulate energy expenditure, suppressing intake, thereby increasing weight loss, leading to reduced body fat accumulation. In conclusion, consumption of MCT-containing foods may contribute to control of body weight and should be recommended as a part of weight loss strategies.

Phytosterols. R.J. Ostlund, Washington University in St. Louis, USA

Enzymatic Production of Betapol™ and Other Structured Lipids. C.C. Akoh, University of Georgia, Athens, GA, USA

Betapol™ (Loders Croklaan) was developed to mimic the structure and composition of human milk fat (HMF) for use in infant formula formulations. It was produced by enzymatic modification of vegetable oils by sn-1, 3-specific lipase. Betapol contains high levels of palmitic acid at the sn-2 position and oleic or unsaturated fatty acids at the sn-1, 3-positions. The aim was to improve the absorption of calcium, fat, and to soften infant stools after breast feeding. Various studies have reported the syntheses and fatty acid compositions of Betapol and similar HMF analogs. Infant formula fat analogs can be synthesized to include unsaturated eicospentaenoic (EPA, 20:5 n-3), docosahexaenoic (DHA, 22:6 n-3), γ-linolenic (GLA, 18:3 n-6), and stearidonic (SDA, 18:4 n-3) acids for their bioactive or physiological functions. Vegetable oils and palmitic acid-containing fats are also used as part of the substrates. These structured fats are referred to as structured lipids (SLs). Amaranth, hazelnut, canola, and modified soybean oils have been used to prepare infant milk fat analogs. SLs lipids can also be prepared to replace trans fats in margarine, shortenings, and spreads formulations for their functionality in foods. Palm and palm kernel oils as well as stearic acid-containing fats are often part of the substrates.

Nutritional Characteristics of Diacylglycerol Oil. T. Yanagita, Saga University, Saga, Japan

Dietary lipids have been recognized as contributory factors in the development and the prevention of metabolic syndromes and cardiovascular risk clustering. Therefore, it is important to know what kinds of lipids are adequate for our health.Diacylglycerol(DAG) occur naturally as a minor component, present up to about 10% of various vegetable oils. Dietary DAG, particularly 1,3-DAG, has been reported to have metabolic characteristics distinct from dietary triacylglycerol(TAG). Studies in animals and human demonstrated biological effects of dietary DAG such as lowered postprandial blood triglyceride level, body weight/fat reductions, and increased fat metabolism. One of the explanations was generated by animal studies showing that lymphatic transport of chylomicron after 1,3-DAG ingestion was significantly delayed and reduced than TAG ingestion, presumably as a result of poor re-esterification of FA onto either 1-monoacylglycerol or glycerol, major digestive products from 1,3-DAG. In addition, repeated ingestion of DAG increased activities of beta-oxidation of liver and small intestine, suggesting the mechanism responsible for the body weight/fat reduction on a DAG diet. Having these

clinical evidence and mechanism of actions, DAG may be promising aid for preventing the diseases associated with the obesity and metabolic syndrome when used in place of vegetable oil in regular diet.

EPA and DHA-rich Oils. N. Salem, A. Ryan, Martek Biosciences, USA

Algal oil-derived DHA has now been fed to tens of millions of babies in infant formula in the US and throughout the world the source of which is the dinoflagellate C. Cohnii. Food and supplement oil derived from the Thraustochytrid Schizochytrium sp. are also widely used and contain both DHA and DPAn-6 fatty acids. More recently, a different strain of Schizochytrium sp. has been developed that is enriched in both DHA and EPA and may be considered a "vegetarian fish oil". A quick search of the literature indicates over 16000 research reports about EPA or DHA. It is well established now that DHA is critical for normal brain and retinal functions and deficiency leads to a loss of a variety of functions. On the other had, supplementation of the diet with EPA and DHA leads to increased bloodstream and tissue levels of these lipids and provides a health benefit in a growing number of organ systems and diseases. This may be largely the result of the modern diet departing from a higher n-3 and lower n-6 traditional diet in many cultures. Several important mechanisms have been found for DHA involving the modulation of G-protein coupled receptor signaling, decreased apoptosis, modulation of protein expression and synthesis of bioactive molecules similar to anandamides or resolvins.

α-Tocotrienol: The Natural Vitamin E Against Stroke. Chandan K. Sen, Ohio State University, OH, USA

Following failed clinical trials testing α-tocopherol (TCP), interest in other naturally occurring forms of vitamin E is sharply rising. Meta-analyses of clinical trials testing the efficacy of vitamin E in human health suffer from a blind spot because they fail to recognize that α-TCP, the only form of vitamin E tested in such trials, represent one-eighth of the natural vitamin E family. Our laboratory has demonstrated that neuroprotection by α-tocotrienol (TCT) at nanomolar concentration represents the most potent functional property of the entire vitamin E family. Neuroprotective as well as hypocholesterolemic properties of α-TCT make it a good candidate for nutrition-based intervention in people at high risk for stroke. Transient ischemic attack (TIA), or ministroke, serves as a sentinel warning sign for high-risk stroke patients. Prophylactic stroke therapy therefore provides an opportunity for intervention in TIA patients prior to a major stroke event. The current state of evidence warrant clinical assessment of α-TCT in TIA patients. Furthermore, α-TCT is a nutrient that is GRAS certified by the US FDA and is not a drug with potential side effects. Thus, α-TCT may be considered as a preventive nutritional countermeasure for people at high risk for stroke. Supported by NIH NS42617.

Lutein and Zeaxanthin: Dietary Sources, Bioavailability and Bioactivity. M.G. Ferruzzi, Purdue University, West Lafayette, IN, USA

Carotenoids, a family of hydrophobic pigments abundant in fruits, vegetables and grains, have drawn significant attention in recent years due to their association with several health benefits. While the provitamin A activity of carotenoids including β-carotene is well documented, non-

provitamin A carotenoids including lutein and zeaxanthin have been increasingly studied for their health promoting activities including: antioxidant activity, cardiovascular health, eye health, skin health and neuroprotection. With the potential for a significant role in health promotion, interest in content, bioavailability and biological activities of these pigments from foods has intensified. This lecture will focus on dietary sources of lutein and zeaxanthin and will discuss factors impacting bioavailability of these pigments. Additionally, the impact of food formulation and processing on stability, absorption and metabolism of these bioactive pigments will be described. Finally, tissue distribution and specific bioactivities will be discussed in the context of proposed mechanism of disease prevention. A better understanding of natural sources of lutein and zeaxanthin, as well as factors affecting their bioavailability and activity is critical to development and assessment of products for specific health benefits.

BIO 3.1: Biocatalysis II

Chair(s): C.T. Hou, USDA, ARS, NCAUR, USA; and K. Miyashita, Hokkaido University, Japan

Separation of Nutraceutical Glycolipids. Makoto Suzuki[1], Tomoki Takahashi[1], Shiomi Watanabe[1], Leo Tanaka[2], Yuko Haruta[2], Makoto Shiota[2], Masashi Hosokawa[1], Kazuo Miyashita[1], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan, [2]Megmilk Snow Brand Co., Ltd., Kawagoe, Saitama, Japan

In the present study we demonstrate effective concentration of milk sphingolipids and their nutraceutical functionality. Lipid concentrated-butter serum (LC-BS) containing 59.3 % lipids was prepared from butter serum through acidic treatment and ultrafiltration. After removal of neutral lipids from LC-BS with hexane extraction, the residual polar lipids were subjected to hydrolysis with lipase. Phospholipids were hydrolyzed to fatty acids and glycerol-phosphocholine/ethanolamine, while milk sphingolipids were not hydrolyzed. As there were much difference in polarity of three fractions obtained after lipase treatment, namely sphingolipids, free fatty acids, and glycerol-phosphocholine/ethanolamine, each fraction was easily separated. Animal experiments indicated that milk sphingolipids significantly reduced the plasma cholesterol, hepatic total cholesterol and triacyglycerol levels. Although there was little difference in the fatty acid composition of all dietary lipids, significant decrease (P

Synthesis of 1,3-dicapryloyl-2-docosahexaenoylglycerol by a Lipase Reaction. Y. Yamauachi-Sato, S. Negishi, The Nisshin OilliO Group, Ltd., Yokosuka, Kanagawa, Japan

A two-step consecutive synthetic method for the production of symmetrical, structured lipids by a combination of non-selective and sn-1,3 regio-selective ester-exchange reactions was investigated. In the first step, triacylglycerols with unspecifically substituted DHA chain were obtained by reacting tricapryloylglycerol (CCC) with ethyl docosahexanoate (EtDHA) using the lipase QLM, followed by removing the ethyl ester and CCC by molecular distillation. In the second step, sn-1,3 regio-selective ester-exchange was achieved by reacting the resulting triacylglycerols with ethyl caprylate (EtC) using the lipase Novozyme 435, followed by distillation of the ethyl ester and CCC to give sn-1,3-dicapryloyl-sn-2-docosahexaenoylglycerol (CDC). The distillates CCC, EtDHA, and EtC could be recycled repeatedly to produce CDC as the substrate for the consecutive ester-exchange reaction. The present method is considered to

meet the requirements for industrial utilization, in which simplicity in scale-up, high yields, compact reaction system and minimal formation of side-products, are important factors.

Plant *PAHs* Complement the *pah1Δ* Mutation in *Saccharomyces cerevisiae*. E. Mietkiewska[1], R.M.P. Siloto[1], J. Dewald[2], S. Shah[3], D.N. Brindley[2], R.J. Weselake[1], [1]Department of Agricultural, Food and Nutritional Science; University of Alberta, Edmonton, Alberta, Canada, [2]Department of Biochemistry; University of Alberta, Edmonton, Alberta, Canada, [3]Plant Biotechnology, Alberta Innovates-Technology Futures, Vegreville, Alberta, Canada

Phosphatidate phosphatase-1 (PAP1) catalyzes the hydrolysis of phosphatidate to produce *sn*-1,2-diacylglycerol, the immediate precursor of triacylglycerol. In the current work, we studied Arabidopsis *PAH1* (At3g09560) and *PAH2* (At5g42870) and two novel *Brassica napus PAHs*: *PAH1A* (HQ11385) and *PAH1B* (HQ113854). To gain insights into their function, the coding regions of Arabidopsis and *B. napus PAHs* were linked to a *GAL1* promoter in the yeast expression vector pYES2/NT and transformed into the *Saccharomyces cerevisiae pah1Δ* strain. Recombinant expression studies confirmed that these homologous *PAHs* encode enzymes with PAP activity and can rescue different phenotypes exhibited by the yeast *pah1Δ* strain, such as temperature growth sensitivity and atypical neutral lipid composition. Using a yeast system, we examined the role of the putative catalytic motif DXDXT and other conserved residues through mutational analysis. Mutants in the C-LIP domain exhibited significant loss of PAP activity which was reflected by their limited ability to complement different phenotypes of *pah1Δ*. Sub-cellular localization studies using a Green Fluorescent Protein (GFP) fusion protein showed that Arabidopsis PAH1 is mainly present in the cytoplasm of yeast cells. Oleic acid treatment, however, led to GFP fluorescence in the nucleus.

Enzymatic Analysis of Linoleic Acid Transformation to Conjugated Linoleic Acid in *Lactobacillus plantarum*. Shigenobu Kishino[1,2], Kenzo Yokozeki[1], Sakayu Shimizu[2], Jun Ogawa[2], [1]Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Applied Microbiology, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

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. 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 produces two CLA isomers, i.e., *cis*-9,*trans*-11 and *trans*-9,*trans*-11-CLA, from linoleic acid with 10-hydroxy-12-octadecenoic acids (18:1) as intermediates [1)]. We tried to identify the enzymes involved in CLA production from linoleic acid of *L. plantarum* AKU 1009a and three proteins (CLA-HY, CLA-DH, and CLA-DC) were obtained from the cell-free extracts. CLA-HY was in membrane fraction, and CLA-DH and CLA-DC were in soluble fraction. Functional properties of CLA-HY were examined in detail. CLA-HY catalyzed the reaction forming 10-hydroxy-*cis*-12-18:1 from linoleic acid in the presence of FAD. CLA-HY showed strict substrate specificity toward free form of C18 unsaturated fatty acids with Δ9 double bonds in cis configuration.Reference:1)Kishino, S. et al. (2009) *Appl. Microbiol. Biotechnol.*, 84, 87-97.

Microbial Conversion of Arachidonic Acid to Arachidonyl Alcohol by a New Microorganism. Toshihiro Nagao, Motohiro Shizuma, Yomi Watanabe, Yuji Shimada, Osaka Municipal Technical Research Institute, 1-6-50 Morinomiya, Joto-ku, Osaka, Japan

Aeromonas hydrophia converted rapeseed, safflower, and linseed oils to wax esters. The wax esters included several rare unsaturated fatty alcohols which were produced by microbial-mediated reduction of the corresponding fatty acids. Since reducion of unsaturated fatty acids to unsaturated fatty alcohols is difficult reaction by industrial chemical methods, the strain may facilitate introduction of new bioprocess for producing unsaturated fatty alcohols, especially fatty alcohols harboring more than two double bonds. We thus aimed to convert functional arachidonic acid to arachidonyl alcohol. First, we screened suitable microorganism. Single cell oil (arachidonic acid content, 40 wt%) was employed for the cultivation (oil addition, 50 mg/mL-culture), and we found that strain No. 476-2, that belonged to different genus with *Aeromonas*, most effectively converted arachidonic acid to arachidonyl alcohol. Time course of the cultivation of the strain showed that 2.2 mg/mL-culture of arachidonyl alcohol was produced after 4 days. GC-MS, NMR, and FT-IR analysis showed that the resulting product was 5*cis*,8*cis*,11*cis*,14*cis*-arachidonyl alcohol. These results showed that the strain effectively converted carboxyl group of arachidonic acid to hydroxyl group without alterations of the double bond position and conformation.

Enzymatic Synthesis and Characterization of *trans*-free Structured Margarine Fat Analog using Stearidonic Acid-enriched Soybean Oil and High Stearate Soybean Oil. G. Pande, Casimir C. Akoh, The University of Georgia, Athens, GA, USA

Enzymatic synthesis of *trans*-free structured margarine fat analog from stearidonic acid (SDA)-enriched soybean oil and high stearate soybean oil was optimized using response surface methodology (RSM). The independent variables considered were substrate molar ratio (2-5), temperature (50-65 °C), time (6-22 h), and enzymes (Lipozyme® TLIM and Novozym® 435). Two dependent variables were studies namely, mol% of stearic acid incorporation and mol% SDA content. A good-fit model was constructed using regression analysis with backward elimination and verified by a chi-square test. Desirable and optimal products composition were achieved at 50 °C, 18 h, 1:2, using Lipozyme TLIM, with 14.23 mol% stearic acid and 7.03 mol% SDA in the product and at 58 °C, 20 h, 1:2, using Novozym 435, with 15.10 mol% stearic acid and 5.20 mol% SDA. Using optimal conditions, structured lipids (SLs) were synthesized in a 1 L stir-batch reactor and free fatty acids were removed by short-path distillation. SLs were characterized using GC for fatty acid profile, *sn*-2 positional analysis, HPLC for triacylglycerol profile, x-ray diffraction for polymorphism, NMR for solid fat content, and DSC for melting/crystallization profiles.

Development and Application of Oleaginous Filamentous Fungus *Mortierella alpina*. Akinori Ando[1], Yuka Tanaka[1], Hiroshi Kikukawa[1], Tomoyo Okuda[1], Eiji Sakuradani[1], Jun Shima[1], Jun Ogawa[1], Sakayu Shimizu[2,1], [1]Kyoto University, kyoto-shi, Japan, [2]Kyoto Gakuen University, Kameoka-shi, 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-vector system for *M. alpina* 1S-4 was developed by means of molecular breeding for improving and modifying PUFAs productivity and composition. Thus far, two transformation systems for this fungus have been established with uracil auxotrophy and antibiotic carboxin resistance as homologous selectable markers, respectively. In this study, we demonstrate the transformation system and application in this fungus. In particular, we evaluated the eicosapentaenoic acid (EPA, 20:5n-3) production, which was an end product of n-3 fatty acids synthesized in *M. alpina* 1S-4, by homologous overexpression of ω3-desaturase gene catalyzing conversion of n-6 fatty acids to n-3 fatty acids and that of Δ12-desaturase gene catalyzing conversion of n-9 fatty acids to n-6 fatty acids.

WEDNESDAY

MORNING

BIO 4 / S&D 4: Biobased Materials: Surfactants, Polymers, and Enzymes in Green Cleaning

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

Enhanced Stabilization of Cloudy O/W Emulsions with a Blend of Gum Arabic/Whey Protein Isolate. N. Garti, M. Klein, A. Aserin, I. Svitov, Casali Institute for Applied Chemistry, Hebrew University, Jerusalem, Israel

Cloudy emulsions are oil-in-water (O/W) emulsions normally prepared as concentrates which are diluted into the final beverages. The cloudy emulsions provide flavor, color and cloud (turbidity) to the soft drinks. These systems are stabilized by emulsifiers, by amphiphilic polysaccharides.Whey protein isolate (WPI) and gum Arabic (GA) are natural biopolymers. From previous work, we learned that mixing WPI and GA together in an aqueous phase may result in the formation of a charge complex soluble in water. The charge complex, we believe, has a potential to serve as natural emulsifier for cloudy emulsions.The aim of our work is to stabilize an O/W emulsions by the blend of the two natural biopolymers of WPI and GA at selected conditions (pH, oil type, preparation protocol, etc). These cloudy emulsions must be stable and should fulfill the requirements of the beverages.We concluded that stable emulsions were obtained from 3:1 wt ratio of WPI:GA. The emulsions shows better stability than emulsions stabilized by GA or WPI alone. The droplets size were smaller then 1 μm and did not change significantly during a month of aging . The emulsion with the highest turbidity was composed of 3 wt% of WPI:GA (3:1) and 20 wt% Canola oil.

Multifunctional Green Surfactants from Crops. S.R. Jadhav, G. John, The City College of The City University of New York, New York, NY, USA

Sugars and fatty acids are ideal natural precursors to develop value-added chemicals and serve as sustainable alternatives to petroleum-based products. Current research exemplifies the approach by synthesizing novel amphiphiles, which are versatile oil gelators with varied industrial

applications. Short chain fatty acids (C_4-C_{10}) and sugar alcohols (mannitol and sorbitol) were chosen as crop-based precursors. Enzyme mediated regioselecetive transesterification, was employed to obtain amphiphilic sugar dialkanoates. These non-toxic and biodegradable amphiphiles, specifically C_8-derivatives, exhibited unprecedented gelation in crude oil fractions, edible oil and liquid pheromones. Structure-property relationship was thoroughly evaluated to fundamentally understand the gelation mechanism. The amphiphiles also exhibited preferential gelation of oil from oil-water mixture; such phase-selective gelation was utilized to demonstrate an environmentally benign method to clean-up oil spills. The vegetable oil gelation property was exploited as healthy alternative to existing oil structuring methods used in food industries. The amphiphiles were also utilized to compose gel-based controlled release devices for pheromones, which find application in agricultural pest management.

Clickable Sophorolipid Surfactants. J.A. Zerkowski, D.K.Y. Solaiman, USDA, ARS, ERRC, Wyndmoor, PA, USA

This presentation will report our recent progress in modifying the structures of sophorolipids to make them more water soluble. The methodology that we have been exploring includes the azide/alkyne "click" reaction and ruthenium-catalyzed olefin metathesis. Both of these routes can be used to attach hydrophilic groups to sophorolipids. The linkages that result are not readily hydrolyzable, which is a distinction from previous modified sophorolipids, where ester bonds were used to append charged units. Preliminary results suggest that hydroxyproline is a versatile moiety for introducing a zwitterion at the carbohydrate headgroup of a glycolipid. Progress toward constructing gemini sophorolipids using similar methods will also be described.

Production and Interfacial Characterization of New Types of Glycolipid Biosurfactants. Dai Kitamoto, Tokuma Fukuoka, Tomotake Morita, Tomohiro Imura, AIST, Japan

Biosurfactants (BS) produced by a variety of microorganisms show unique properties compared to petroleum-based surfactants. Among BS, glycolipid-type BS such as mannosylerythritol lipids (MEL) and sophorose lipids (SL) are the most promising, due to the high productivity from vegetable oils or carbohydrates as renewable resources [1]. MEL are efficiently produced by Pseudozyma yeasts, and show versatile interfacial and biochemical actions. SL are produced by Candida yeasts, and now used as a washing agent. It is thus of interest to broaden the structural variety of these promising BS, and to clarify the structure-function relationship. Recently, we tried to synthesize new types of BS from di-acetylated MEL and SL, and obtained their deacetylated derivatives. We also obtained mono-acetylated glucose lipid and glucose lipid from di-acetylated SL. We then characterize the interfacial properties of these glycolipid BS. These new BS showed the similar surface activity to that of the starting materials, but indicated different aqueous phase behaviors. These results demonstrated that the acetyl group on the sugar moiety is likely to play more important role to direct the self-assembling manner of glycolipid BS.[1] D. Kitamoto et al., Curr. Opi. Colloid Interf. Sci., 14, 315-328 (2009).

Rhamnolipid Production and Purification. M. Sodagari, Y. Chen, J. L. Lilly, N. M. Pinzon, L.-K. Ju, The University of Akron, Akron, OH, USA

Rhamnolipids are unique biosurfactants produced by bacteria. Rhamnolipids are extremely

effective in emulsifying/solubilizing hydrocarbons, for clean-up of oil spills, bioremediation of petroleum-contaminated sites, and enhanced oil recovery. They have also been proposed as pesticides, as the source of rhamnose, and for several medical applications. Rhamnolipid production is complicated by the highly foaming nature of the fermentation broth when aerated to provide oxygen for cell respiration. We have investigated the rhamnolipid production by Pseudomonas aeruginosa fermentation under aerobic, microaerobic and anoxic denitrifying conditions. Effects of different carbon substrates have also been evaluated. The behaviors and challenges observed will be summarized in this presentation. About 70 g/L of rhamnolipids can be produced at 0.35 g/L-h. We have also been evaluating and developing purification methods for future large-scale operations at lower costs. The results from our recent and on-going work in rhamnolipid production and purification will be presented. If time permits, we will also briefly describe some new applications of rhamnolipids.

Development of Bio-detergent using RSPO-certified Sustainable Palm Oil. Yoshihiko Hirata, GlenLelyn Quan, Keisuke Igarashi, Taro Furuta, Saraya Co. Ltd., Kashiwara, Osaka, Japan

Biodiversity loss which accelerated in these years due to overexploitation of natural resources causes a rapid increase in the worldwide demand for bio-detergents, which are not only ready-biodegradable but also created from renewable and sustainable materials. In line with this, a certification system evaluated by a third-party organization has been in operation to help build a sustainable world. In the plant oil market, certified sustainable palm oil (CSPO) which is certified by Roundtable on Sustainable Palm Oil (RSPO) has been available since the end of 2008. On the other hand, we started the research of bio-based surfactants (biosurfactants, BS) for practical use as bio-detergents since 1998 and developed the automatic-dishwashing detergent containing sophorolipid, which is one of the glycolipid BSs. Sophorolipid may replace propylene oxide-ethylene oxide block copolymer surfactants which have low-foaming and high-washing ability but have poor or inconsistent biodegradability. Currently, ten bio-detergents are commercialized for domestic and industrial use and in healthcare facilities in Japan. This report contains our developed methods both for large yield fermentative production and simple purification of SL derived from CSPO resulting to a laundry bio-detergent marked with RSPO certification label.

Improved Bioreactor Design and a Mathematic Model for Solvent–Free Lipase–Catalyzed Synthesis of Saccharide–Fatty Acid Ester in Suspension Media. Ran Ye, Douglas G. Hayes, Department of Biosystems Engineering and Soil Science, the University of Tennessee, Knoxville, TN, USA

Saccharide–fatty acid esters, biodegradable, and nonionic biobased surfactants derived from cheap agricultural renewable sources, possess excellent properties for emulsification resulting in their wide use in foods, cosmetics, and pharmaceuticals. Previously, we have conducted esterification utilizing stable 10–200 μm suspensions of saccharide in solvent–free media. However, this approach has suffered from the stoppage of the recirculation to reform suspensions for several hours at a time. Recently, the operation and design of the bioreactor system have been improved, starting with the mixture of oleic acid/ fructose oleate 75/25 w/w, using the fed–batch addition of saccharide stirring at 65°C, containing an in–line filter to remove large suspended

particles. After filtration, the suspension media is sent to a vessel where water is controlled at the optimal level of ~0.4 wt % via vacuum pressure and nitrogen gas bubbling and subsequently the effluent is sent to a PBBR, and recirculated to the stirred reservoir. The bioreactor system yielded a final conversion of 85% (0.195mmol h^{-1} g^{-1}) with ~90% of monoester without further purification. A mathematical model is currently under development to describe the time course of reaction utilizing material balances and a Ping–Pong Bi Bi kinetic model.

Interfacial Properties of Surfactant-like Extracts from Waste Biomass. E.J. Acosta, F.Y. Garcia-Becerra, M. Baxter, D.G. Allen, University of Toronto, Toronto, ON, Canada

Surfactant-like materials extracted from waste biomass contain a wide range of chemical species that include proteins, polysaccharides, lipids, phospholipids and humic material. In this article we discuss how the composition and properties of the extract are impacted by the extraction pH and our current understanding of the relationship between composition of the extract and its ability to lower surface and interfacial tension. We will also discuss the impact of waste source/composition on the surface activity of the resulting extract. This discussion will lead to a series of scenarios where the extraction of waste biomass could be economical and might represent an advantage over petroleum-based products.

****Cancelled** Synthesis and Properties of Esterquats Derived from Rice Bran Fatty Acids and Triethanolamine.** V.K. Tyagi, Harcourt Butler Technological Institute, India

****Cancelled** Surface-active and Performance Properties of Alkyl Polyglycoside (APG) Surfactants Derived from Fatty Alcohols.** V.K. Tyagi, Harcourt Butler Technological Instit

Recent Developments in Cleaning with Cellulase Enzymes. N.J. Lant[1], A. Calvimontes[2], V. Dutschk[3], S.G. Patterson[1], [1]Procter & Gamble Technical Centres Ltd, Newcastle upon Tyne, United Kingdom, [2]Leibniz Institute of Polymer Research, Dresden, Germany, [3]University of Twente, Enschede, The Netherlands

Cleaning cellulases selectively modify the amorphous regions of cotton, resulting in durable laundry detergency benefits in the areas of soil release and anti-redeposition. In recent years, new cleaning cellulase enzymes such as Celluclean® and advances in formulation science have led to improved products with new consumer benefits and reduced dependence on commodity chemicals, for example in helping to enable the removal of builders such as sodium tripolyphosphate. Analysis of the morphology and topometry of fabric surfaces has improved our understanding of the effects of cleaning cellulase on textiles, helping to explain the observed detergency benefits as a function of changes in macro-, meso- and micro-porosity. Carboxymethylcellulose (CMC) is currently used as an anti-redeposition agent in many laundry detergents, also acting through a surface modification mechanism, and this polymer is susceptible to hydrolysis by cleaning cellulases. Molecular optimisation of CMC to synergise with emerging cleaning cellulase technology has resulted in new enzyme/polymer fabric surface

modification systems with improved performance.

Breakthrough Enzyme Technology for Laundry Soap Bars. Nelson Prieto[1], Cheila Cavanholi[1], Michael Bullock[1], Christian Wieth[2], Peter Klindt-Mogensen[2], Yang Zaizhou[3], [1]Novozymes North America, Franklinton, NC, USA, [2]Novozymes A/S, Bagsvaerd, Denmark, [3]Novozymes China, Beijing, China

Enzymes deliver unique cleaning benefits in various laundry applications but have not been used widely in laundry soap bars because it is difficult to stabilize them in a typical laundry soap bar matrix. An enzymatic breakthrough innovation has been developed for laundry soap bars that introduces a major performance advancement in this product category. Enzymes have been added and stabilized in diverse laundry soap bars enabling increased cleaning benefits without adversely affecting other key characteristics and delivering a product preferred by consumers. Results from diverse wash performance, physical properties, and stability tests were confirmed with consumer studies. The results will be presented demonstrating the benefits the breakthrough enzyme technology delivers in laundry soap bars.

BIO 4.1: Plant Lipid Biotechnology

Chair(s): D. Hildebrand, University of Kentucky, USA; and J. Shockey, ARS, USDA, NCAUR, USA

New Omega-3 and Monounsaturated Fatty Acid Resources. David Hildebrand, Runzhi Li, Yongmei Wu, Watchareewan Jamboonsri, Tim Phillips, University of Kentucky, Lexington, KY, USA

There is a growing need for additional ω3 fatty acid resources. Among high ω3 sources *Salvia hispanica* has very high production potential with limited inputs but seed set is limited in areas where plant growth is particularly well adapted. We have developed many new lines that can produce seed in autumn in much of the US with good agronomic and nutritional characteristics. Some lines have as much as ~ 65% α-linolenic acid and are among the highest dietary sources of healthful fiber, antioxidants and calcium. *S. hispanica* seed yields of 1,000 ? 2,000 kg/ha can be achieved with the new lines with little or no fertilizer and no weed control. However yields can be enhanced with modest fertilization and some herbicide combinations. Like most plants total plant dry matter accumulation can be greatly enhanced with modest soil nitrogen increases but seed shattering and plant lodging needs to be bred out for maximum seed yield potential to be achieved. Among monounsaturated fatty acids, palmitoleic acid ($16:1\Delta^9$) is particularly healthful. Selective transfer of 16:1 into seed oil triacylglycerol (TAG) is found in a high natural accumulator, *Macfadyena unguis-cati*,. Acyl-CoA: diacylglycerol acyltransferases (DGATs) as well as phosphatidylcholine: diacylglycerol acyltransferase (PDAT) all show expression patterns consistent with roles in accumulation of 16:1 in seed oil TAG.

Oilseed Metabolic Engineering: Gene Discovery and Analysis of Factors that Affect Triacylglycerol Synthesis and Accumulation in Transgenic Plants. J. Shockey[1], X. Li[2], H. Cao[1], A. Ullah[1], K. Sethumadhavan[1], S. Boone[1], T. Klasson[1], J. Dyer[3], E. Cahoon[2], [1]USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA, [2]Center for Plant Science

Innovation, Department of Biochemistry, University of Nebraska, Lincoln, NE, USA, [3]USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ, USA

Oils of the seeds of bitter gourd (Momordica charantia) and tung tree (Vernicia fordii) contain approximately 60% and 80%, respectively, of α-eleostearic acid (18:3Δ9cis,11trans,13trans), an unusual conjugated trieneoic fatty acid with valuable drying properties. Our laboratories study tung and bitter gourd as model systems for the production of industrially useful oils. Some of the important components of these pathways have been identified; other essential components of a successful gene stacking ensemble remain to be found. Genes currently in hand are being expressed and characterized in a number of different transgenic organisms. Additional improvements aimed at optimizing the effectiveness of extant transgenes currently will also be discussed, including changes that may improve transcriptional timing, translational efficiency, and protein stability. Collectively, our recent efforts to identify new components of the TAG biosynthetic pathway, and to characterize changes in existing enzymatic activities that either positively or negatively affect the synthesis and accumulation of eleostearic acid in transgenic plants and microbes will be presented.

Modifying the Oil Content of Soybean Seeds. Anthony Kinney, Knut Meyer, DuPont, Wilmington, DE, USA

Soybean seeds, which contain approximately 40% protein and 20% oil, are an important feed ingredient as well as an important source of crude vegetable oil. Our overall goal is to generate soybean seed quality traits that provide added value for feed, fuel, industrial and food applications. The first generation of soybean quality trait research was based upon improving the oxidative stability of soybean oil, which resulted in the development and commercialization of high oleic soybeans (Plenish). This new oil can address a wide range of food and industrial applications. More recently our research efforts have been directed at increasing the oil content of soybean seeds. Genes that control flux through triglyceride biosynthesis, as well as genes that control the redirection of carbon flux into triglyceride biosynthesis, have been used to significantly increase the oil content of the soybean seed without any reduction in protein content or seed weight.

New Soybean Oil Developments at Monsanto. Toni Voelker, Monsanto, Davis, CA, USA

To make soybean oil suitable for frying applications, the polyunsaturates can be reduced via chemical hydrogenation, and this partially hydrogenated soybean oil was used for many food products in the US. Monsanto has produced a reduced-saturates, less polyunsaturated soybean oil naturally through a modification of soy fatty acid biosynthesis in developing seeds: Vistive® Gold. Vistive® Gold allows to eliminate trans fats and significantly lower saturated fat content in fried foods without sacrificing flavor quality. In fact, frying tests done with chicken and French fries have shown no change in taste or texture while delivering all the benefits of improved cooking oil, including reduced saturated fat and zero trans fat. Vistive® Gold soybeans have advanced to Phase IV (pre-launch), the final step within the R&D pipeline. Soymega™ stearidonic acid (SDA) soybean oil, formerly known as SDA Omega-3 was developed to serve as a source of improved omega-3 fatty acids. Soymega™ is an optimal omega-3 source be cause it can be added to a broad variety of food products. It has stearidonic acid (SDA), which readily

converts to eicosapentaenoic acid (EPA), one of several omega-3 fatty acids used by the body when consumed. Updates will be given for these two new vegetable oils.

Toward Production of Castor Oil in Transgenic Oilseeds and How Arabidopsis Fights Back. P.D. Bates, J. Browse, Washington State University, Pullman, WA, USA

The Castor plant accumulates up to 90% of its seed fatty acids (FA) as the hydroxylated FA (HFA) ricinoleate. Ricinoleate is a common industrial HFA used to make a variety of polymers, lubricants and biofuels. However, the Castor plant is highly toxic and has agronomic features that are not suitable for large scale production in the US. Therefore, we are engineering Arabidopsis to produce Castor type oils as a model for engineering other oilseed crops. Expression of the Castor FA hydroxylase (FAH12) alone produces ~17% HFA in seed triacylglycerol (TAG). FAH12 produces HFA on the sn-2 position of the membrane lipid phosphatidylcholine (PC) however, the HFA need to accumulate on the three backbone positions of TAG. We hypothesize that Arabidopsis does not efficiently remove HFA from PC and incorporate it into TAG. Support for this hypothesis has been demonstrated by coexpression of FAH12 with HFA specific TAG biosynthetic enzymes from Castor. Castor versions of either phospholipid:diacylglycerol acyltransferase (PDAT) or acyl-CoA:diacylglycerol acyltransferase (DGAT) increased HFA content to ~28% of seed FA. Analysis of both FA and glycerol backbone flux into TAG have demonstrated some of the metabolic responses to HFA production that limit HFA accumulation in Arabidopsis. These experiments may lead to new engineering strategies for accumulation of HFA in high yielding transgenic oilseeds.

Biotechnological Development of Camelina as a Biofuel and Biolubricant Crop. E.B. Cahoon, H.T. Nguyen, J.E. Collins-Silva, T.J. Nazarenus, R.E. Cahoon, A.K. Reddy, University of Nebraska-Lincoln, Lincoln, NE, USA

Camelina sativa (false flax) is an emerging Brassicaceae oilseed crop in the Great Plains and Pacific Northwest of the United States. The growing interest in camelina is due largely to its potential for biodiesel production in geographic areas that are not well-suited for soybean cultivation. We are also exploring the use of camelina as a platform for the production of lubricants, biofuels, and high-value industrial oils. Camelina is a good candidate to fill this niche because it is not widely grown in the US for food use. In addition, genetic transformation of camelina can be achieved by a simple floral vacuum infiltration of agrobacterium. With this method, metabolic engineering of camelina can be conducted in a rapid and non-labor intensive manner. To this end, we have initiated a biotechnological pipeline to generate camelina lines with improved fuel and lubricant properties (e.g. enhanced oxidative stability). We are also developing field capacity to evaluate the agronomic properties of engineered lines and to generate sufficient quantities of seeds for oil functionality testing. Single and multi-gene oil traits engineered to date include high oleic acid, high vitamin E, high saturated fatty acids, and wax esters, an alternative fatty acid storage form.

Metabolic Engineering of Seeds can Achieve Levels of ω-7 Fatty Acids Comparable to the Highest Levels Found in Natural Plant Sources. Tam H. Nguyen[1,3], Girish Mishra[3], Edgar Whittle[3], Scott A. Bevan[2], Ann Owens-Merlo[2], Terence A. Walsh[2], Mark S. Pidkowich[3], Edgar Cahoon[1], John Shanklin[3], [1]University of Nebraska Lincoln, Lincoln, NE, USA, [2]Dow

AgroSciences, Indianapolis, IN, USA, [3]Brookhaven National Laboratory, Upton, NY, USA

Plant oils containing ω-7 fatty acids (FA) (palmitoleic 16:1 Δ9 and cis-vaccenic 18:1Δ11) have potential as sustainable feedstocks for producing industrially important octene via metathesis chemistry. Engineering plants to produce seeds that accumulate high levels of any unusual FA has been an elusive goal. We have achieved high levels of ω-7 FA accumulation by systematic metabolic engineering of plant. A plastidial 16:0-ACP desaturase has been engineered to convert 16:0 to 16:1Δ9 with specificity >100-fold that of naturally-occurring paralogs such as that from Doxantha unguis-cati L. Expressing this engineered enzyme (Com25) in seeds increased ω-7 FA accumulation from

Identification of Important Amino Acid Residues for Plant Diacylglycerol Acyltransferase-1 Activity. Rodrigo M.P. Siloto, Randall J. Weselake, University of Alberta, Edmonton, AB, Canada

In oilseeds, the activity of acyl-CoA:diacylglycerol acyltransferase (DGAT) plays a major role in controlling the synthesis of triacylglycerol (TAG). DGAT genes have been overexpressed in oleaginous crops as a means to increase seed oil production. Even though DGATs have applications in oilseed biotechnology, little is known about the residues involved in their catalytic activity. Here we used site-saturation mutagenesis to explore the role of particular residues on the activity of a plant DGAT1. This method consists of creating libraries of all twenty naturally occurring amino acid substitutions at a specific position. We generated libraries containing all possible substitutions in several conserved residues of castor bean DGAT1. These libraries were expressed in yeast and screened using a Nile red fluorescence method. Initial results indicated that the conserved motifs HX_3D, HX_4D and RX_3E previously proposed to compose the catalytic site are non-essential for DGAT1 activity. The conserved prolines P^{220}, P^{363} and P^{456} are also non-essential but correlate with higher enzyme activity. Interestingly the substitution $C^{221}L$ increased substantially the amount of TAG formed by yeast cells demonstrating the utility of this work for the development of improved enzyme variants.

Putative Regulation of Brassica napus diacylglycerol acyltransferase 1 (DGAT1) Mediated by its N-terminal Domain. M.S. Greer, M. Truksa, N. Sharma, W. Deng, R.J. Weselake, Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada

Diacylglycerol acyltransferase (DGAT) catalyzes the acylation of sn-1,2 diacylglycerol to produce triacylglycerol , the primary component of seed oil. Up-regulating DGAT transcription during seed development in plants has been shown to increase seed oil content. It is possible that post-transcriptional events may also affect the final DGAT activity during seed development. Detailed studies of the biochemical regulation of DGAT, however, have been hindered by the hydrophobic nature of this integral membrane protein. Recently, we identified four gene homologs encoding variants of Brassica napus DGAT1 (BnDGAT1).The predominant differences between these enzyme forms resides in their N-terminal regions. This region is less conserved, unnecessary for catalysis and more hydrophilic than the remaining residues. Production of recombinant chimeric enzymes in yeast, with interchanged N-terminal domains, however, demonstrated that certain forms of this domain support twice the oil accumulation in

vivo as compared to the others. Western blots of protein extracts from these yeast cultures suggest the increased DGAT activity is due to increased enzyme accumulation. Thus, the cellular production of BnDGAT1 may be regulated at the protein level by the nature of its N-terminal region.

Role of Phospholipases in Storage Lipid Production. Xuemin Wang[1,2], Geliang Wang[1,2], Maoyin Li[1,2], Amanda Tawfall[1,2], Brian Fanella[1,2], [1]University of Missouri, St. Louis, St. Louis, MO 63121, USA, [2]Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

In most plant species, storage lipids accumulate primarily in the form of triacylglycerol (TAG) stored in oil bodies in seeds. Phospholipids play pivotal roles in TAG production. The most common phospholipid phosphatidylcholine (PC) serves as a substrate for fatty acid desaturation and other modifications and can also provide diacylglycerol (DAG) directly for TAG synthesis. The simplest, minor phopsholipid phosphatidic acid (PA) provides DAG for PC and TAG biosynthesis. PA also plays a role in acyl trafficking between the endoplasmic reticulum and plastids, where TAG and fatty acids are produced. In addition, PA is a class of important mediators in cell signaling, membrane trafficking, and cytoskeleton rearrangement. We have been investigating the roles of different classes of phospholipases, which hydrolyze PC to PA or DAG, in seed lipid metabolism and accumulation. The results indicate that phospholipases play important roles in regulating seed oil production by affecting the metabolism of key intermediates in TAG biosynthesis and by modulating cellular regulation of TAG accumulation.

AFTERNOON

BIO 5: General Biotechnology

Chair(s): D. Solaiman, USDA, ARS, NCAUR; and L.K. Ju, University of Akron, USA

Lipase-catalyzed Hydrolysis of Salmon Oil to Concentrate Omega-3 PUFA: Modeling and Optimization of the Process. D. Kahveci, X. Xu, Department of Molecular Biology, Aarhus University, Denmark

Long chain omega 3 polyunsaturated fatty acids (PUFA) have many beneficial effects, such as treatment and/or prevention of cancer and cardiovascular diseases; development of nervous tissues; improvement of immune system; treatment of mental diseases; etc. Concentrated form of such fatty acids is a commonly preferred dietary supplement, due to the reduced oil intake. Enzymatic processes, compared to conventional methods, are considered superior in fish oil industry, since they are effective under mild reaction conditions, and their fatty acid selectivity improves the omega 3 PUFA yield. The study aimed to concentrate omega 3 PUFA in oil obtained from by-products of farmed salmon. Response surface methodology was applied to optimize the reaction conditions. Temperature, time, enzyme load, and water-to-oil ratio were investigated. Total omega 3 PUFA content, EPA/DHA ratio in the product and oleic acid (OA)/total PUFA in released free fatty acids (FFA) were the responses of interest. EPA/DHA ratio determined a balanced omega 3 PUFA content, while OA/total PUFA in FFA was a measure of the selectivity of the reaction. Results showed that all three of the second-order

polynomial models adequately fitted the experimental data. The models generated were predictive when reaction was scaled up by ~67-fold.

Encapsulation of Omega-3 Oils. A. Sundararajan, Martek Biosciences Corporation, Winchester, KY, USA

Omega-3 oils contain long chain polyunsaturated fatty acids (LC-PUFA) including DHA and EPA that are highly susceptible to oxidation. Measures taken to protect these PUFAs from oxidation include minimizing exposure to oxygen, heat, light and transition metals during the manufacturing process and during product handling & storage, use of antioxidants, encapsulation and low temperature storage. Microencapsulation is an enabling technology that allows these sensitive ingredients to be physically enveloped in a protective matrix or wall material. This physical barrier can be beneficial in different ways: protect against oxidation, flavor masking, minimize ingredient interactions, and controlled release. This presentation will provide a background on omega-3 oils highlighting the need to encapsulate them. Various encapsulation technologies and methods used to assess the quality of the resulting powder will be reviewed. Challenges to omega-3 fortification of foods and beverages will be discussed along with key considerations and strategies in choosing an encapsulation technology for omega-3 oils.

A Biodiesel Feedstock of Triacylglycerides from Acid Hydrolysate of Switchgrass and Woodchips. Guochang Zhang, Todd French, Rafael Hernandez, William Holmes, Mississippi State University, Mississippi State, MS, USA

Biodiesel is a displacement fuel for traditional petroleum-derived diesel. Unfortunately biodiesel is an expensive fuel due in large part to the high cost of feedstocks. Oils derived from lignocellulosic biomass could potentially be a cheap source of biodiesel. The objective of this investigation is to determine the feasibility of using activated sludges from wastewater treatment facilities in State of Mississippi and Rhodotorula glutinis (ATCC 15125) to produce triacylglycerides from acid hydrolysates of switchgrass and woodchips as a biodiesel feedstock. Both switchgrass and woodchips were hydrolyzed with sulfuric acid. Total sugar concentration in acid hydrolysates of switchgrass and woodchips are determined by HPLC-ELSD method to be 55.36g.l-1, 38.0 g.l-1, respectively. In the experiments, both cell growth and lipid accumulation were investigated using the media made of acid hydrolysates. The lipid content in the cells was measured by gravimetrical Bligh & Dyer extraction. The majority contents of triacylglycerides were characterized by high temperature gas chromatography to be of C16~C20. This study demonstrated that it is feasible for oleaginous microorganisms to convert lignocellulosic sugars to triglycerides as a renewable biodiesel feedstock.

Continuous Production of Biodiesel from Corn Oil in a Supercritical Carbon Dioxide Bioreactor. O.N. Ciftci , F. Temelli, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Optimized production of biodiesel using green technology, based on supercritical carbon dioxide ($SCCO_2$) technology coupled with enzyme technology was investigated. Continuous production of biodiesel (fatty acid methyl esters; FAMEs) from corn oil was studied in a $SCCO_2$ bioreactor using immobilized lipase (Novozym 435) as catalyst. Response surface methodology based on

central composite rotatable design was employed to investigate and optimize the reaction conditions: pressure (11-35 MPa), temperature (35-63 °C), substrate mole ratio (molar ratio of methanol to corn oil; 1-9) and CO_2 flow rate (0.4-3.6 L/min, measured at ambient conditions). A predictive quadratic model was developed for the FAME content. Increasing substrate mole ratio increased the FAME content, whereas increasing pressure decreased the FAME content. Higher conversions were obtained at lower and higher CO_2 flow rates compared to moderate flow rates. Similarly, reactions at lower and higher temperatures resulted in higher FAME contents compared to reactions at moderate temperatures. The optimal reaction conditions generated from the model for the maximum FAME content were 19.4 MPa, 62.9 °C, 7.03 substrate mole ratio and 0.72 L/min CO_2 flow rate. The optimum predicted FAME content was 98.9% and the actual value was 93.3 ± 1.1%.

Lipid Characterization of Certain Microalgaes with Biofuel Application. G. Wang, T. Wang, Iowa State University, Ames, IA, USA

A full procedure with cell wall breaking, lipid fractionation, and characterization of neutral and polar lipids in two oleoginous microalgae were established. Our hypothesis is the presence of non-TAG lipid fractions, such as phytosterols, FFA, polar lipids, and even ask content could affect the efficiency and cost of making biodisel from microalgae. Our works focus on a full characterization of lipid fractions for two oleoginous microalgal species and an establishment of a principle in preparing algal oils for biofuel application.

Use of Algae to Modify Waste Oil for Biodiesel Production. M. Hosseini, L.-K. Ju, The University of Akron, Akron, OH, USA

The scale and sustainability of biodiesel production is limited by the availability of cheap feedstock. Waste cooking oil and some other potential oil/lipid sources have high free fatty acid contents that prevent their direct transesterification to biodiesel by the more effective and economic alkali-catalyzed process. We have recently shown that certain high-lipid algae species grow rapidly on oil and fatty acids, and decrease the acid value of the unconsumed waste cooking oil, presumably because of the preferential consumption of free fatty acids over glycerides. This process can potentially serve to pretreat/upgrade the oils with high free fatty acid contents for easier subsequent biodiesel production. In addition, the high-lipid alga was found to have much more hydrophobic cell surface than common microorganisms. Once the mixing used in the culture system was stopped, a predominant majority of the algal cells would attach to the rising oil droplets and become concentrated in a layer at the oil-water interface. Algae cells could be easily harvested. The yield and properties of biodiesel prepared from the oil and lipids collected from the algae process will be compared with those from the untreated waste cooking oil.

****Cancelled** Quantitative Synthesis of Tyrosyl Oleate Catalyzed by Two Immobilized Candida antarctica Lipases in Solvent-free Medium.** C.F. Torres, CIAL, Spain

Biotechnology Posters

Chair(s): R. Ashby, ARS, USDA, NCAUR, USA

****Cancelled** Manufacture of Biodiesel via Transesterification Reaction from Capparis Spinosa Oil Seed with Methanol and Basic Catalyst.**
K. Tahvildari, Azad University, Iran

Evaluation of *Rhizopus oryzae* Lipase Selectivity in Presence of Short/medium Chain Fatty Acids.
M. Pérignon[1], J. Lecomte[1], M. Pina[1], A. Renault[2], C. Simonneau-Deve[2], P. Villeneuve[1],
[1]CIRAD, UMR IATE, Montpellier, France, [2]St Hubert, St Hubert, Rungis, France

Nutritional impact and fatty acids (FA) bioavailability are governed both by the overall FA composition and the stereospecific distribution of FA in triacylglycerols (TAG) molecules. The determination of FA regiodistribution on TAG requires accurate methods that can be employed on a wide range of oils and fats substrates. The most widely used method for regiodistribution analysis is an enzymatic one, involving the use of pancreatic lipase. However, due to lipase selectivity, this method knows some restrictions for fats containing medium and short chain fatty acids. Alternative methods suffer various drawbacks like using toxic chemical (Grignard reagent) or expensive tools (13C NMR or LC-MS). Thus, there is still a need in identifying new lipases with limited acylselectivity that could be an alternative to pancreatic lipase for the sn-2 position analysis of fats containing short/medium chain FA.The aim of the current study was to evaluate the selectivity of Rhizopus oryzae lipase in presence of short/medium chain fatty acids in partial hydrolysis conditions used for regiodistribution analysis. Structured triacylglycerols containing eight carbons chain length fatty acids in sn-2 position were chemically synthesized using DCC/DMAP coupling agent and purification steps by flash-chromatography. Final product showed very high purity and was used as substrate for 1,3-selectivity evaluation. Typoselectivity was assessed by investigating partial hydrolysis of equimolar blend of homogeneous TAG containing eight or twelve carbons chain length fatty acids.

Reduction of Free Fatty Acids in Crude Palm Oil via Trifluoromethanesulfonic Acid.
Adeeb Hayyan[1], Mohd Ali Hashim[1], Farouq S. Mjalli[2], Maan Hayyan[1], Inas M. AlNashef[3],
[1]Department of Chemical Engineering, University of Malaya, Kuala Lumpur, Malaysia,
[2]Petroleum & Chemical Engineering Department, Sultan Qaboos University, Muscat, Oman,
[3]Chemical Engineering Department, King Saud University, Riyadh, Saudi Arabia

Malaysia, as one of the biggest palm oil producers and exporters in the world, produces large amount of crude palm oil (CPO) from its mills. Due to the high free fatty acids content (FFA) in CPO, the alkali catalyzed transesterification to produce biodiesel gives low biodiesel yield because FFA reacts with alkali to produce soap. To resolve this problem, esterification reaction was used to convert FFA to fatty acid methyl ester (FAME). In this study, trifluoromethanesulfonic acid (TfOH) was used in the pre-treatment of CPO by esterification process. The purpose of pre-treatment process was to reduce the FFA content in CPO to a

minimum level for biodiesel production. Esterification process of CPO was carried out to evaluate TfOH as a super acid catalyst. The optimum conditions showed that the FFA of CPO reduced from 8.3% to less than 0.5% FFA and the yield of treated CPO and conversion of FFA to FAME were 95% and 97% respectively.

Methyl Ester Epoxidation from Crude Canola Oil by Biocatalysis: An Alternative for Reduction of Environmental Impacts in Epoxides Production.
Rosana de Cassia de Souza Schneider, Manuella Schneider, Fernanda Bock, Andre Luiz Klafke, Jorge André Ribas Moraes, Santa Cruz do Sul University, Santa Cruz do Sul, Rio Grande do Sul, Brazil

The epoxides are used in chemistry industry and can be obtained by different methods, chemical and enzymatic. The use of lipases as biocatalysts in perhydrolysis reactions, originated from carboxylic acids with peracids can provide oxidation capacity. The lipase is capable of hydrolyzing triacylglycerol turning them into free fatty acids, and these, in turn, in the presence of hydrogen peroxide leads to the formation of peracids. Thus, this study has applied the chemo-enzymatic epoxidation, which had been developed to sunflower oil, in order to epoxide methyl esters of canola oil with Novozyme 435 lipase, well as has evaluated the environmental impacts of this process compared to chemical method. The chemical method was with preformed peracetic acid and was required purification steps involving precipitation, washing and rota-evaporation. Through chemo-enzymatic method, after reaction, was carried out the phases separation and rota-evaporation. As result we obtained a conversion â?¥ 99% in oxiranes rings. It was possible to identify that environmental impacts related to chemo-enzymatic method were lower than with chemical method because it is possible to reuse the solvent and lipase and it does not need purification. CNPq, PUICvol-UNISC, FINEP, SCT-RS, FAP-UNISC

Biocatalyzed Modification of Coconut Oil to Contain Polyunsaturated Fatty Acids.
L.J. Pham[1], A.J. Pham[2], [1]BIOTECH, U.P., Los Banos, The Philippines, [2]Mississippi State University, USA

Structured Lipidsâ?? application in clinical nutrition is established.However,their application and use in consumer products is just emerging.Several researches have been undertaken to upgrade coconut oil.One of these is restructuring through biotechnology for products demanded by the consuming public. Production of structured coconut oil containing omega-3 and 6 fatty acids was carried out with Lipozyme IM 20 and with three non-lauric oils namely fish oil,pili pulp oil and cashew nut oil.Non-lauric oil concentrates were prepared with Candida cylindraceae lipase.The omega- 3 content in the triacylglycerol fraction of the fish oil concentrates was found to increase from 25.36% to 74.99%.Enrichment of Eicosapentaenoic acid(EPA) was from 12.10% to 34.39%. This was lower than that of Docosahexaenoic acid(DHA) which had an enrichment of 8.06% to 31.02%.Triacylglycerol molecular species indicated that structured coconut oil was prepared. In the coconut-fish oil combination,the lower molecular weight species(PN 28-32) had a percentage change from 12.49% to 23.45% while the intermediate molecular species changed from 46.66% to 55.35 % indicating interchanges mediated by enzymes forming new triacylglycerols which incorporated the polyunsaturated fatty acids.

TAG Biosynthetic Enzymes and Palmitoleic Acid (16:1Δ9) Accumulation in Seed Oils of

Macadamia and Cat's Claw.
R. Li, D. Hildebrand, University of Kentucky, Lexington, KY, USA

Palmitoleic acid (16:1Δ9) is a mono unsaturated fatty acid, which is very beneficial in health and industrial application. There is an increasing need for this important fatty acid in both food and medical industry. Two of naturally high accumulators for this high-valued fatty acid are Macademia (Macadamia integrifolia) with 30% palmitoleic acid in seed oil and cat?s claw (Macfadyena unguis-cati) with 80% 16:1Δ9 + 18:1Δ11in its vine. Our previous report showed most of palmitoleic acid is accumulated in TAG fraction. Here, we present data to describe the cloning and functional characterization of DGAT1, DGAT2 and PDAT, three TAG biosynthetic enzymes from developing seeds of those two source plants. Lipid class distribution in subcellular parts and different tissues and three gene expression files were also investigated. The present results provide new insights into understanding of palmitoleic acid biosynthesis and new strategies to develop engineered commercial oilseeds producing high level of palmitoleic acid for health and industrial applications.

Cocoa Butter Equivalent from Pili Pulp Oil by Lipase Catalyzed Modification.
L.J. Pham, D.B. Libunao, R.D Tambalo, BIOTECH, U.P., Los Banos, The Philippines

A cocoa butter equivalent (CBE) can be prepared from Pili(Canarium ovatum Engl.) Pulp oil by lipase catalyzed interesterification.The objective of this research was to investigate the structure modification of pili pulp oil to a CBE using Lipozyme IM-20 and a Biotech Lipase from Rhizopus delemar.The study showed that the composition of the CBE was affected by acyl donor sources,substrate ratio,initial water of enzyme,reaction time and enzyme loading.The best reaction conditions for CBE production were substrate ratio (pili pulp oil:palmitic acid:stearic acid) at 1:2:3;water activity of enzyme at 0.11;reaction time at 4h;reaction temperature at 45 C and 20% enzyme loadingMelting point of the CBE was at 32-37 C.

Modification of the Chicken and Goose Fat via Enzymatic Interesterification with Vegetable Oils.
M. Kostecka, B. Kowalski, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

In this study, chicken/goose fat and vegetable oil (rapeseed oil and sunflower oil) mixture was enzymatically interesterified to produce fats with an enriched UFA and oleic/linoleic acid content. Because of saturated fatty acids are located mainly in the outer, and unsaturated fatty acids in the inner positions of the chicken/goose fat triacylglycerols (TAG) molecules, the commercial preparation Lipozyme RM IM containing 1,3-regioselective lipase was used as the catalyst. Properties of the enzymatically modified products were compared. The following parameters were assayed in the starting mixture and crude products after interesterification: acid value (titration method), polar and nonpolar fraction content (column chromatography), oxidative stability (Rancimat test and PDSC). Fatty acid composition of TAG and TAG molecular species were determined from the GC results. The sn-2 and sn-1,3 distributions of fatty acids in the TAG were determined using modified Brockerhoff method.

Purification of Catfish Oil Biodiesel Using an Adsorption Process.
K. Mis Solval, S. Sathivel, Louisiana State University Agricultural Center, Baton Rouge, LA,

USA

The quality of biodiesel from crude catfish oil, and the effect of the neutralization and the adsorption procedures on the physicochemical properties of catfish biodiesel were investigated. Unpurified catfish oil (CO) was recovered from catfish viscera; neutralized (NCO) with 20% NaOH solution; transesterified (BNCO) using methyl alcohol (1:6 molar ratio) and NaOH (1% w/w); the resulting FAMEs were purified by 10% (w/w) activated earth (PBNCO). The samples were evaluated for yield, FAMEs, free fatty acids (FFA), peroxides value (PV), moisture, bulk density, cloud point, flash point, free and total glycerin, color, rheological properties, and minerals. The yield was significantly (P

Tocochromanols and γ–Oryzanol –Associated Components of Rice Bran and Rice Bran Oil Bodies.

Nantaprapa Nantiyakul, Greg Tucker, David Gray, University of Nottingham, Sutton Bonington, Leicestershire, United Kingdom

Oil bodies are observed mainly in the sub–aleurone and aleurone layer of brown rice as revealed by transmission electron microscopy. Oil bodies recovered and washed in 9M urea were significantly (*P*

Epoxidation of Copaiba Oleoresin, Sesquiterpene and Diterpene Acids by Chemical and Enzymatic Process - Lipase Promiscuity.

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The copaiba oleoresin is obtained from the trunk of the tree of Leguminosae-Caesalpinoideae family, easily found in the tropics in Latin America, mainly in the Amazon region. There are many applications in medicine described in the literature such as analgesic, cicatrizing, bactericide, anti-helminthes, anti-inflammatory, anti-cancer and gastro-protector. The oleoresin is transparent and varies in color from light gold to dark brown, depending on the ratio of resin(diterpene acids) to essential oil(sesquiterpenes). The sesquiterpene fraction contains a mixture of beta-trans-cariophylene (in more percentage), alpha-copaene and alpha-humulene. The resin contains the diterpene acids as copalic and hardwickiic. The enzymatic promiscuity concept involves the possibility that one active site of an enzyme can catalyze several different chemical reactions. The lipases are able to catalyze the epoxidation reactions by a chemo-enzymatic mechanism. This presentation will describe the epoxidation of copaiba oleoresin, sesquiterpene and diterpene acid fractions, as well as the epoxidation of beta-trans-cariophylene and alpha-humulene standards by conventional chemical and chemo-enzymatic processes, using the lipase promiscuity.

Crystallization and Melting Behaviour of Structured Lipids Produced with Lard and Soybean Oil by Enzymatic Interesterification in a Continuous Packed Bed Reactor.

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The interesterification is currently the most important physical-chemical modification process of oils and fats, and can be used for the production of structured lipids similar to human milk fat. This study aimed to analyze the thermal properties of structured lipids of artificial human milk fat synthesized by continuous enzymatic interesterification from mixtures of lard and soybean oil in various proportions. The interesterification was conducted in packed bed bioreactor with sn-1,3 specific lipase (Lipozyme TL IM, Novozymes). Differential Scanning Calorimetry (DSC) was used to determine the thermal properties of mixtures of lard and soybean oil before and after enzymatic interesterification. In general, the dilution of lard with addition of soybean oil decreased the values of enthalpy and onset and peak temperatures of crystallization and melting. Interesterification affected lard and mixtures thereof, forming new triacylglycerols and new interactions between them, since it promoted significant changes in profiles of the thermograms obtained by DSC. On the other hand, the values of Avrami exponent n remained almost constant, indicating the same morphology of crystallization.

Designing Oilseeds for Tomorrow's Markets: Summary of a Genome Canada Project Aimed at Improving Canola Meal.

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The Designing Oilseeds for Tomorrow?s Markets (DOTM) project was a four-year Genome Canada initiative (ending Dec 31, 2010) aimed at increasing the value of canola meal. While canola is primarily cultivated for its premium oil, the seeds are also naturally rich in protein, vitamins, and minerals. However, canola meal is an under-appreciated and poorly utilized commodity consistently discounted by about 40% relative to soybean meal over the last 20 years. This is mainly due to the presence of anti-nutritional factors (ANFs) associated with canola meal that detract from the full value of the meal. These ANFs include phytate, sinapine, fibre, and glucosinolates. DOTM?s research aimed to decrease ANFs in canola meal, increase health-promoting substances (carotenoids, xanthophylls, glucoraphanin), and identify the genetic mechanisms underlying an elite low fibre-high oil yellow-seeded line. DOTM has generated new canola germplasm with reduced glucosinolates, phytate and sinapine and increased carotenoids, xanthophylls, and glucoraphanin. We have identified one locus responsible for the yellow seeded phenotype and defined the physiological differences in the seed that account for its low fibre phenotype. We also have shown the improved performance of the yellow seeded line in poultry feeding trials relative to traditional black-seeded canola.

Lipase-Catalyzed Modification of Canola Oil with Caprylic Acid.

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Lipase-catalyzed acidolysis of canola oil with caprylic acid was performed to produce structured lipids. Six commercial lipases from different sources were screened for their ability to incorporate the caprylic acid into the canola oil. The positional distribution of FA on the glycerol backbone of unmodified oil and oil modified by Lipozyme RM IM, Lipozyme TL IM and Novozyme 435 were analyzed by ultra HPLC (UPLC). The results showed that Lipozyme RM IM from Rhizomucor miehei resulted in the highest caprylic acid incorporation and the lowest acyl migration so it was used in further studied. Reaction parameters studied included substrate mole ratio, enzyme load, reaction time and temperature. Incorporation of caprylic acid was higher when reactions were carried with 10% lipase of the total weight of substrates at a mole ratio of oil to caprylic acid of 1:4. The optimal time course and temperature for synthesis structured lipids were 15h and 50-60°C. Possible triacylglycerol species and physical properties of the SL product obtained at relative optimal conditions were characterized.

Solvent-Free Lipase-Catalyzed Synthesis of Saccharide-Fatty Acid Ester in Suspension Media: Effect of Acyl Donors and Acceptors.

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Saccharide-fatty acid esters, biodegradable, environmentally-friendly, and nonionic biobased surfactants, are widely applied in the foods, pharmaceutical and cosmetics industries. In our previous study, they have been successfully synthesized by immobilized Rhizomucor miehei lipase (Lipozyme ® IM, Novozymes, Franklinton, NC, USA) at 53oC utilizing 10-200 micron-sized solvent-free suspensions of saccharide in a packed bed bioreactor (PBBR) system with strong selectivity toward monoester. The by-product, water, was efficiently removed via free evaporation supplemented by the combination of vacuum and nitrogen gas at ~0.4 wt %, the latterÃ¢â?¬â?¢s introduction into the bioreactor system initiated optimally into the time course of reaction, The optimized bioreactor system achieved ~93 % conversion of fructose oleate (85% of which was monooleate) and a productivity of 0.297 mmol h-1 g-1 at 65oC utilizing suspension solvent-free media in PBBR or stirred tank bioreactor system (STBR). This approach was extended to different acyl acceptors (fructose, sucrose, xylose, glucose) and donors (lauric acid, myristic acid, caprylic acid and oleic acid).

Enzymatic Modification of Freshwater Catfish Oil for Human Milk Fat Substitutes.

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Human milk fat substitute (HMFS) was prepared by Lipozyme RM IM-catalyzed acidolysis of China freshwater Catfish oil (rich in 30% palmitic acids and more than 50% of palmitic is located at sn-2 position) and fatty acids obtained from cocoa nut oil, high oleic sunflower oil and soybean oil, in a solvent-free system using packed-bed reactor. The interesterified products were purified by molecular distillation and the resulting HMFS (more than 65% palmitic acid is located at sn-2 position) was evaluated for physical and chemical properties. The aim of this study was to effectively modify China freshwater Catfish oil via lipase catalysis for use as an alternative fat source for infant formula and other infant products.

Purification of Palm Oil-Based Fatty Acid Methyl Ester using Deep Eutectic Solvents.

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Production of biodiesel has attracted the academic as well as the industrial communities in recent years. One of the most serious obstacles in using biodiesel as an alternative fuel is the intricate and costly purification processes involved in its production. The difficulties in the separation of glycerol and other reaction mixtures necessitate the development of new competent low cost separation processes. In the present work, Choline Chloride-based deep eutectic solvents (DES) were used as solvents for extracting glycerol from the palm oil- based biodiesel, particularly fatty acid methyl ester (FAME). The laboratory-scale purification experiments established the viability of this technique as the purified biodiesel fulfilled the EN 14214 and ASTM D6751 standard specifications for biodiesel fuel in terms of glycerol content.

Simultaneous Spectrophotometric and Chemometric Determination of Oleic, Linoleic, and Linolenic Fatty Acids in Vegetable Oils.

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Following the success of simultaneously quantifying molar concentrations of lipids in human serum using a mature, patented reagent system selective to the -CH=CH-CH_2 group, the application of the assay is extended to vegetable oil samples. A central composite design of training set (n=30) and simplex lattice design of prediction set (n=10) matrices were generated containing oleic, linoleic, and linolenic fatty acid components in chloroform solutions. Neural network (NN), principal component regression (PCR), partial least squares (PLS1 and PLS2), and K-matrix (KM) algorithms were utilized for the deconvolution of the spectra. Results show that the root mean square error of prediction compared quite equally well for PCR, PLS1, and PLS2 algorithms for the three components, with these algorithms outperforming NN and KM. In a study involving sunflower, soybean, safflower, olive, and flaxseed oils, the same algorithms (PCR, PLS1, and PLS2) performed quite well in such unknown sets.

Nanometric Brazilian Clays as Catalyst for Obtaining Biodiesel.

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The known versatility of bentonite clays, non-expensive and environmentally friendly materials, is available in clay based catalyst promoting, in general, gain in yield and selectivity. In this work we have studied four Brazilian bentonite clays in their form in raw clay and clay-supported zinc oxide as catalyst systems. The objective was to compare the performance of these catalyst systems to conversion of soy oil into biodiesel. Firstly the pristine clays were treated with alcohol/water solution (1:1) followed by addition of sodium/ammonium hydroxide solution, and

the resulting clay suspension was heated in microwave oven for 15 min at 100°C. After centrifuged and dried the clays were supported with zinc oxide. The raw clays clay-supported zinc oxide catalysts were characterized by X-ray diffraction, scanning electronic microscopy, X-ray fluorescence, thermal analysis, IV spectrometry. The catalyst reaction of soy oil to biodiesel was realized under mild reaction conditions in microwave oven, at 90°C for 15 min. The catalytic performance was studied. Materials with high selectivity and yield were obtained.

Cyclopropane Fatty Acid Accumulation in Plant Oil.

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There is an enormous need to meet a greater share of our material needs from renewable resources. Among the major targets are plant derived-lubricants. Plant oils can currently be used as automobile engine oils but do not meet automotive manufacturer?s specifications due to the poor oxidative stability of the unsaturated fatty acids. Solutions to this dilemma include converting the double bonds commonly found in plant oils into cyclopropyl groups. Such groups can have adequate low temperature fluidity and lubricity plus the needed very high oxidative stability. Various organisms are capable of synthesizing and in some cases accumulating cyclopropyl groups in seed oil triacylglyceride (TAG). In developing lychee seeds, we found that a cyclopropane fatty acid (CFA) accumulates in TAG. We have transformed soybean with a CFA synthase gene producing TAG and other lipids with a cyclopropyl group. The rather even distribution of CFA in various lipid classes in developing seeds of the transgenic soybeans indicated that the CFA was not selectively incorporated into TAG as often observed in other unique fatty acid accumulating plants such as vernolic acid in Vernonia seeds. The positional analysis of CFA in TAG as well as phosphatidyl choline is under way to evaluate the role of various enzymes involved in TAG synthesis.

Synthesis of Triacylglycerol Containing CLA by Lipase–catalyzed Esterification under Vacuum.

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Synthesis of CLA (conjugated linoleic acid)-enriched TAG (triacylglycerol) was carried out by direct esterification of glycerol and CLA using Novozym 435 (*Candida antarctica* lipase). Original CLA, which is used as a substrate, consisted mainly of 9*c*,12*t*-CLA of 35.6 wt% and 10*t*,12*c*-CLA of 36.6 wt%, and the water formed during the reaction was controlled by vacuum. The highest yield (90 wt%) of TAG was obtained at 60 °C, enzyme loading of 10 wt% based on the total substrates and a vacuum of 3 mmHg. The fatty acid composition of TAG indicated that 10*t*,12*c*-CLA was incorporated more quickly and to a greater extent than 9*c*,11*t*-CLA in all positions, as well as the *sn*-2 position of glycerol. Moreover, it was found that the content of CLA at the *sn*-2 position was lower than at *sn*-1 and *sn*-3. Consequently, it was determined that the direct esterification of glycerol and CLA can be successfully achieved by Novozym 435, and this lipase has selectivity for 10t,12c-CLA and individual fatty acids have a preferential position on the backbone of glycerol.

Production of Monoacylglycerol Containing Pinolenic Acid Using Lipase–mediated Reaction.

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Pinolenic acid (PLA) is one of the Δ5-unsaturated polymethylene interrupted fatty acids which is contained mainly in pine nut oil (PNO). It was reported that the pine nut oil containing PLA may works as an appetite suppressant through an increasing effect on satiety hormones. Hence, if monoacylglycerol (MAG) containing PLA could be produced, it would be a novel MAG as nutraceutical emulsifier. MAG was synthesized in a solvent-free system by lipase-mediated esterification of glycerol and fatty acids derived from PNO. Several lipases were screened to observe their ability for producing the MAG containing PLA. Among the lipases tested, Lipase G from *Penicillium camembertii* was selected for further optimization. The effects of parameters, such as temperature, water content, molar ratio, and enzyme loading were observed. When vacuum was applied after initial reaction conducted under no vacuum, the purity of MAG was improved. Unpredictably, Lipase G showed an activity for producing of MAG at freezing temperatures, even though the reaction time of long term was necessary.

Synthesis of Symmetrical Triacylglycerol Containing Pinolenic Acid at *sn*–2 Position in Packed Bed Reactor by Lipase–catalyzed Acidolysis.

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Structured lipid was synthesized in a packed bed reactor (PBR) by acidolysis of capric acid and a modified pine nut oil (MPNO) using an immobilized lipase (Lipozyme RM IM) from *Rhizomucor miehei*. The MPNO, which was enriched pinolenic acid at *sn*-2 of triacylglycerol (TAG), was prepared by lipase-catalyzed acyl migration from pine nut oil. The acidolysis reaction was conducted in PBR at 60°C with different residence time. The effect of water content on the incorporation was investigated between 0.005 and 0.3% based on total substrate. The incorporation of capric acid in MPNO increased as the water content increased. An acyl migration, which capric acid was migrated from sn-1,3 positions to sn-2 position, was also accompanied with increased water content. The effect of molar ratio (MPNO to capric acid) on the incorporation was investigated between 1:3 and 1:9. The incorporation of capric acid and the yield of the desired TAG increased as molar ratio increased up to 1:5, but those decreased when the molar ratio further increased up to 1:9. Optimal water content in reaction mixture and molar ratio of substrates were 0.4 % and 1:5, respectively.

Influence of Seed Storage Protein Gene Composition on Structural Features of Major Crucifer Proteins: Protein Secondary Structure Study of *Arabidopsis thaliana*.

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Crucifer seeds are rich in oil and protein. Proteins of crucifers are primarily cruciferin and napin. Cruciferin, a hexameric protein composed of six subunits is encoded by three paralogous genes while napin is encoded by five genes in Arabidopsis. Arabidopsis expressing cruciferin composed of homologus subunits and no napins have been created by knocking out cruciferin and napin expression genes. The new genetic variants produced seeds having similar amount total proteins. High resolution synchrotron-FTIR technique was used for examining secondary structure of these in planta produced protein and retained in seed tissues. Protein secondary structure features of modified Arabidopsis lines showed significant differences in the deconvoluted amide I bands. Wild-type Arabidopsis proteins exhibited higher β-sheet content than α-helices. Cruciferin triple knock out line contained increased levels of turns and disordered structure suggesting the presence of unfolded filler proteins without higher order secondary structures. Napin knock out lines had slightly less β-sheet content than wild type. Secondary structure properties can be related to the protein properties such as accessibility to enzymes thus digestibility.

Enrichment of High Stearidonic Acid (SDA) Soybean Oil with Palmitic Acid at the sn-2 Position by Enzymatic Interesterification for Use as Human Milk Fat Analog.
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Soybean oil enriched with stearidonic acid (SDA) could be added to the diet to increase omega-3 fatty acid intake. SDA soybean oil is rich in stearidonic, linoleic, and palmitic acids. Breast milk fat has over 60% of palmitic acid (PA), by weight, esterified at the sn-2 position to improve absorption of fat and calcium in infants. Enzymatic interesterification of SDA soybean oil and tripalmitin was performed to obtain structured lipids (SLs) with over 60% PA at the sn-2 position of the triacylglycerol. The enzymatic interesterification reactions were catalyzed by Novozym 435 or Lipozyme TL IM under various conditions of time, temperature, and mole ratio of SDA soybean oil to tripalmitin using Response Surface Methodology (RSM). For Lipozyme TL IM, the optimal conditions were 1:2 substrate mole ratio, 50oC for 18 h resulting in 6.82% total SDA and 67.19% PA at the sn-2 position of the SL product. Whereas, Novozym 435 at a 1:2 substrate mole ratio, 50oC for 15.6 h gave 8.01% total SDA and 64.43% PA at the sn-2 position of the SL. The SLs may be useful in producing human milk fat analogs for infant formula with health benefits of the omega-3 FA.