2010 Annual Meeting Abstracts

Biotechnology

MONDAY

MORNING
BIO 1: Sterols I
Chair(s): R. Moreau, USDA, ARS, ERRC, USA; and P.J.H. Jones, University of Manitoba, Canada

The Role of a Disordered Steroid Metabolome in the Elucidation of Sterol and Steroid Biosynthesis and Metabolism. C. Shackleton[1,2], [1]Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK, [2]Children?s Hospital Oakland Research Institute (CHORI), Oakland, CA, USA

In 1937 Butler and Marrian found large amounts of the steroid pregnanetriol in urine from a patient with the adrenogenital syndrome, a cortisol deficiency condition. This introduced the concept of altered production of metabolites as a tool for understanding sterol and steroid biosynthesis. From the cyclized lanosterol to the most downstream product estradiol, there are around 50 steps. Based on a distinctive metabolome clinical disorders have now been attributed to about 6 post-squalene cholesterol (C) biosynthetic steps and 10 en-route to steroid hormones. 20 Years ago it was thought that almost all steroid biosynthetic defects were known but interest rekindled as novel metabolomes were documented. We have been involved in two ?new? steroid disorders, P450 oxidoreductase deficiency (ORD) and apparent cortisone reductase deficiency (ACRD). Interestingly, these result not from synthetic enzyme mutations, but in ancillary ones necessary for co-factor oxido-reduction. Our third interest is Smith-Lemli-Opitz syndrome (SLOS), a C synthesis disorder caused by 7-dehydrocholesterol reductase mutations. The late George Schroepfer, in whose honor this talk is given, contributed greatly to defining the sterol metabolome of this condition. Finding the cause of clinically severe disorders can lead to improved treatments. We are now involved in murine gene therapy studies which, if successful could in the future offer an alternative therapy for this severe condition.

Biological Activity of Phytosterol Glycosides. Richard E. Ostlund, Jr.[1], Xiaobo Lin[1], Susan B. Racette[2], Lina Ma[1], Robin Fitzgerald[1], Catherine L. Anderson Spearie[3], Robert Moreau[4], [1]Division of Endocrinology, Metabolism and Lipid Research, Washington University, St. Louis, MO, USA, [2]Department of Physical Therapy, Washington University, St. Louis, MO, USA, [3]Institute of Clinical and Translational Sciences, Washington University, St. Louis, MO, USA, [4]Agricultural Research Service, USDA, USDA

Of phytosterols contained in common diets, 9-22% are glycosylated. In order to determine whether glycoside forms are bioactive and should be included in phytosterol measurements we conducted both human and animal experiments. Acylated phytosterol glycoside (ASG) was prepared by selective extraction from soy lecithin. In a randomized, crossover design 11 healthy subjects were given 3 single-meal cholesterol absorption tests in random order that contained 30 mg cholesterol-d7 and either no phytosterols, 300 mg phytosterols as esters (PE) or 300 mg phytosterols as ASG. Compared to no addition, ASG reduced cholesterol absorption 37.6 ± 4.8% and PE 30.6 ± 3.9% (both $p<0.001$). Similar results were seen in mice where, in contrast to PE, ASG phytosterols demonstrated little incorporation into plasma or liver sterols. ASG was quantitatively recovered in the stool as deacylated phytosterol glycoside. Cleavage of glycosidic linkages is not required for biological activity of ASG.

Long Term Cholesterol-lowering Efficacy of Phytosterols. I. Demonty, E.A. Trautwein, Unilever Research & Development, Vlaardingen, The Netherlands

The LDL-cholesterol (LDL-C) lowering efficacy of phytosterol (PS) (encompassing plant sterols and stanols)-enriched foods is well established. Meta-analyses of intervention studies confirmed that PS lower LDL-C by about 9% for a 2 g/d dose. Most studies included in meta-analyses were short-term (typically 4-12 wks). Nevertheless, longer-term studies suggest that the LDL-C lowering effect of PS is maintained over time. Two 1-yr studies with spreads enriched

in plant stanols (Miettinen et al 1995) or sterols (Hendriks et al 2003) showed sustained LDL-C reductions over the treatment phase. Plant sterol- and stanol-enriched spreads were recently shown to similarly lower LDL-C in statin users over a 1.5 yr period (De Jong et al, 2008). Additional support for the sustained efficacy of PS is provided by the Portfolio diet studies, where 2 g/d plant sterols in spread combined with soy protein, dietary fibres and almonds were effective over a 1-yr period, with the main LDL-C lowering effect (9%) being due to plant sterols (Jenkins et al 2006, 2008). Observational data (Wolfs et al 2006) and community intervention studies (deJong et al 2007) provide further support to the long-term effectiveness of PS-enriched spreads. Overall, there is sufficient evidence from longer-term human efficacy studies lasting for up to 1.5 yr that the LDL-C lowering effect of PS intake via enriched foods is sustained.

Plant Stanol Ester Enriched Foods Lower LDL-C and Serum Triacylglycerol Concentrations. J. Plat, R.P. Mensink, Maastricht University, Maastricht, The Netherlands

Plant stanol enriched foods are known for lowering serum LDL-C. In an earlier meta-analysis, we found that in subjects with high baseline serum triacylglycerol (TAG) concentrations, plant stanol esters may lower serum TAG. We therefore initiated two placebo-controlled studies in subjects with elevated serum TAG concentrations. In study 1, a 9-weeks placebo-controlled study with a yogurt drink (2.0 g stanols/d) in 18 patients with the metabolic syndrome we found a 28% reduction in serum TAG (P=0.044) in the stanol group vs placebo. Moreover, the number of large TAG-rich VLDL-1 particles was significantly lower, suggesting a reduced hepatic VLDL-1 production underlying the effect on TAG concentrations. In study 2, a 3-weeks placebo-controlled study with margarine (2.5 g stanols/d) in 28 familial combined hyperlipidemia patients we again found a reduction in serum TAG vs placebo (-11%) but only in those with TAG levels > 2.3 mmol/L (P=0.009). This illustrates that effects on TAG can only be shown in subjects with elevated TAG levels. Although the number of VLDL-1 particles was not significantly lowered in study 2, a significant correlation was found between the plant stanol-induced changes in TAG and VLDL-1 particles.We conclude that foods enriched with plant stanols not only lower serum LDL-C, but also TAG levels in subjects with overt hypertriglyceridemia.

The Occurrence and Biological Roles of Steryl Glucosides in Foods, Vegetable Oils, and Biodiesel. R. Moreau, M.J. Haas, Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA, USA

In animals, cholesterol, C27H46O, is the major sterol. Plants usually contain a mixture of several C28 and C29 sterols, which can occur in several forms, including as steryl glucosides (SGs) and acylated steryl glucosides (ASGs). In plants, ASG and SG are components of biological membranes and may be involved in signal transduction. We recently found that in corn ASG is a major lipid in the membrane surrounding the triacylglycerol-storing organelles. In vitro studies with pancreatic enzymes suggested that the fatty acid portion of ASG is hydrolyzed during digestion, but the glycosidic bond appears to remain intact, as recently confirmed by in vivo studies. Although SGs and ASGs are effectively extracted with polar solvents, they are also partially extracted with hexane and are thus detectable in unrefined plant oils. Conventional refining/bleaching/deodorization (RBD) removes most of the ASG and SG, but both are still detected in RBD oils. Biodiesel is produced by converting the triacylglycerols in oils to methyl (or ethyl) esters. Some of the ASG or SG present in plant oils used to make biodiesel will be carried into the biodiesel product. During this process most of the ASG will be converted to SG. If SG remains dissolved in the biodiesel it has no affect, but under certain conditions, especially low temperatures, the SG can precipitate and cause fouling during storage and in diesel engines.

Oxidation of Plant Sterols in the Industrial By-products of Edible Fats and Oils. Paresh Dutta, S.J. Kumari A. Ubhayasekera, Department of Food Science, Swedish University of Agricultural Sciences, SLU, Uppsala, Sweden

In addition to fatty acids, oxidation also affect other molecules with or without double bond in their chemical structures, such as plant sterols (phytosterols) in food lipids or bulk fats and oils. A wide range of phytosterol oxidation products (POPs) can be formed in vegetable fats and oils during the refining steps of degumming, neutralization, bleaching, deodorization, and during food preparation, heating, storage and handling. Heat treatments often at temperature of 250°C and higher are used in the refining steps. A number of valuable by-products are also formed while refining crude fats and oils for human consumption. Some of the by-products are used as ingredient of

animal feed formulation. The quality of the fatty sources in feed formulation is very important in the production of high-quality food of animal origin. We have studied the levels of POPs in various types of by-products e.g. acid oils from chemical refining, acid oils from physical refining, lecithins, oils extracted from exhausted bleaching earth, hydrogenated fats from by-products, and recycled cooking oils from the food chain. A varieties of POPs such as 5,6-epoxyphytostanols, epimers of 7-hydroxy-, and 7-ketophytosterols, were identified and quantified by GC and GC-MS. Large variations in the levels of POPs were observed which was possibly due to the origins and different processing conditions.

Public Perception of Values of Plant Sterols in Foods. J. Cranfield, University of Guelph, Guelph, Ontario, Canada

Increasingly consumers are using food products containing bioactive compounds as a means to enhance their health and well-being. Plant sterol containing foods and natural health products offer an interested example in this respect. Such products are currently available in a number of regions of the worlds, but are still unavailable in a number of countries, despite their proven efficacy in alleviating elevated levels of serum blood cholesterol. This presentation will review the economic and consumer science literature related to consumer understanding, acceptance of use of plant sterol containing food and natural health products. Focus is placed on the motives and barriers to use of these products, the role of health information in shaping acceptance and use, and characterizing the profile of consumers who use these products.

Global Regulations and Health Claims on Phytosterol-containing Functional Foods. Jerzy Zawistowski, Food, Nutrition and Health, University of British Columbia, Vancouver, BC, Canada

Phytosterols represent one of the most intensely and actively researched groups of bioactives in the area of cardiovascular diseases. This group of compounds has been clinically proven to reduce total and LDL blood cholesterol. Based on their efficacy and safety profile, sterols are approved for use in foods in many countries. One of the challenges food formulators face is following current regulations and laws specific to the target market. The regulations have a significant impact on choice of food matrix, number of serving sizes that are used for delivering phytosterols, ingredient and nutritional composition of food products, as well as allowable maximum daily doses. Manufacturer and marketers are responsible for ensuring that a food label and associated advertising material is truthful, non-misleading and substantiated. To market a new sterol-containing food product, the majority of international jurisdictions require a cumbersome dossier containing information regarding food composition, formulation and processing, as well as clinical and safety substantiation. This talk will focus on the regulatory aspect of sterol/stanol containing foods and health claims, their substantiation, and the authorization systems in various countries.

Economic and Societal Benefits of Plant Sterols in Foods. P.J. Jones, Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada

Plant sterols are naturally occurring chemical compounds that have the potential to lower serum LDL-cholesterol levels in humans when fortified into foods. Currently plant sterol fortified foods are not legal in Canada, despite being permitted for sale in over 35 other countries. The health-related and economic savings of introduction of plant sterols into the diets of Canadians has not yet been assessed. The objective of this study was to quantify the potential economic savings which can be attributed to the consumption of plant sterol enriched foods to allow for informed policy decisions surrounding legalization of plant sterols in Canada. A four step economic simulation model involved i) assessment of a sterol intake ?success? rate, ii) estimation of blood cholesterol reduction due to consumption of foods containing plant sterols, iii) determination of the reduction in the incidence of coronary heart disease (CHD) due to decrease in blood cholesterol levels, and iv) calculation of the reduction in costs associated with CHD due to a reduction in the overall incidence of CHD. The model used varying assumptions to provide both conservative and less conservative estimates. The savings in direct health care expenditures and lost human capital was assessed as ranging from $38 million to $2.4 billion. This reduction represents a significant economic saving for Canada. Supported by CAPSIC.

Phytosterols and Phytosteryl Conjugates in Grains. Anna-Maija Lampi[1], Tanja Nurmi[1], Laura Nyström[1,2], Vieno

Piironen , University of Helsinki, Helsinki, Finland, ETH Zurich, Zurich, Switzerland

The aim is to give an overview of phytosterol composition of cereal grains and their fractions, and present data on their steryl conjugates. Phytosterols were included in a diversity screen study (www.healthgrain.org) where 150 wheat lines and 50 other cereals were analyzed for bioactive compounds. There were differences in phytosterol composition among species, and the highest contents were found in rye (1098-1420 μg/g dm). The contents in various wheat types were 670-1187 μg/g dm. The most abundant phytosterol was sitosterol followed by campesterol and stanols. A study on environmental factors on 26 wheat genotypes showed that growing location had a significant effect on phytosterols.Localization of phytosterols in grains was also studied. Dry milling experiments showed that white wheat flour contained only ca 50% of the phytosterols of whole grains whereas the contents in the bran fractions were up to 300%. During sequential debranning highest phytosterol levels were found in the intermediate and aleurone layers. Milling fractions were also characterized for steryl conjugate profiles. Whole grain wheat flour contained 53%, 35% and 12% of free, esterified and glycosylated sterols, while the proportions in rye flour were 42%, 48%, and 12%, respectively. Profiles varied greatly within a grain. In wheat germ oil, the proportion of steryl glycosides was <1% while it increased to 8% in the bran and up to 15% in the refined flour.

BIO 1.2 / PCP 1: Process and Co-products of Biofuel and Industrial Production
Chair(s): K. Liu, USDA, ARS, PWA, USA; D. Solaiman, USDA, ARS, ERRC, USA; J. Wanasundara, Agriculture & Agri-Food Canada, Canada; and H. Wang, Iowa State University, USA

Product Opportunities from Algae. Joel Butler, Joanna Money, Solix Biofuels, Ft. Collins, CO, USA

While much of the recent interest in algae has been driven by its value as a potential fuel, algae has the potential to deliver a large number of additional products including nutraceuticals, food supplements, feed for animals and fish and bioplastics. Some of these products are more valuable than biocrude but their extraction from the algae is complex and therefore their viability as a product is still unclear. This presentation will describe these potential products in more detail and discuss some of the challenges facing algae producers in extracting their value.

In situ Transesterification of Algae for the Production of Fatty Acid Methyl Esters for Use as Biodiesel. M.J. Haas, K.M. Scott, Eastern Regional Research Center, USDA, Wyndmoor, PA USA

There has recently been considerable interest in the use of lipids synthesized by algae as feedstocks for the production of renewable fuels. One possibility is the production of biodiesel, which consists of the simple alkyl esters of fatty acids, from algal acylglycerols. This would typically involve the dewatering of the algal biomass, recovery of its lipid by pressing or solvent extraction, and transesterification of the resulting lipid preparation to form biodiesel. An alternative to this approach, termed 'in situ' transesterification, is to conduct the transesterification reaction directly on the dried algal biomass. This eliminates the need for pressing or solvent extraction steps to recover the oil from the algal mass before transesterification, and could therefore simplify and reduce the cost of biodiesel production. We have investigated this approach for the synthesis of biodiesel from algal oil, and will present the results of this investigation.

Glycerine - A Valuable Biodiesel Coproduct for Fermentation Processes. R.D. Ashby, D.K.Y. Solaiman, T.A. Foglia, USDA, ARS, ERRC, Wyndmoor, PA, USA

One of the impediments in the widespread use of biodiesel is the prohibitive cost involved in removing and/or refining the glycerine coproduct. Attempts to impart value to crude glycerine have resulted in the development of a number of technologies designed to utilize crude glycerine as a precursor for value-added product synthesis. The focus of our research group is the bioproduction of green polymers and surfactants with potential as substitutes for petroleum-based materials. We have focused our attention on the utilization of low-value feedstocks (*e.g.* glycerine, soy molasses) to improve process economics of fermentation-based syntheses. In this presentation, we will discuss our continued efforts to improve product yields of polyhydroxyalkanoates (PHA; bacterial polyesters) and sophorolipids (SL; glycolipid surfactants) from both refined and crude glycerine through manipulation of fermentation parameters and the potential for property modification of the bioproducts based on the absolute content of the crude glycerine. Lastly, we have demonstrated the antibacterial properties of SL towards *Propionibacterium acnes* by using SL:Poly-3-hydroxybutyrate

$(PHB)_{glycerine}$ composite films and showed, through scanning electron microscopy, the physical effects of SL addition on the surface topography and porosity of PHB polymer films.

Advances in Corn Ethanol Enzyme Technology, Effect on DDGS and Opportunities for Animal Feed Industry. M. Hruby, Danisco Animal Nutrition, Woodbury, MN, USA

Enzymes are crucial in production of ethanol from corn and other starch sources. Enzymes facilitate production of sugars used by yeast to yield ethanol through a fermentation process. Enzymes can also function as processing aids to improve throughput, reduce slurry viscosity and salt deposits in the processing equipment, water and the use of other compounds such as acids. Ethanol production in United States comes mainly from dry mill grind plants. Besides ethanol and CO_2 , dry mill grind plants also produce distillers dried grains with solubles (DDGS). DDGS are increasingly counted as economically important products of ethanol production process. A deliberate focus on higher nutrient digestibility, low antinutrient levels and high uniformity of DDGS could result in a significant increase of ethanol plant′s revenues. Targeted focus on DDGS quality can be crucial at times when ethanol production gives lower return on investment. Nutritionists have been using greater quantities of DDGS in animal diets. Understanding the effect of various enzymes used in ethanol production process on DDGS quality and uniformity can provide final users, whether domestic or in export markets, with a valuable information to achieve a greatest economic benefit in production of animal protein when offered specific DDGS to include in animal feeds.

Techno-economic Analysis of Brassica Protein and Co-product Extraction Technologies for Food and Biofuel Applications. Edmund K Mupondwa, Janitha Wanasundara, Bioproducts and Bioprocesses, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada

This study presents a techno-economic analysis of an aqueous extraction process to recover proteins of Brassica oilseeds, focusing on napin (2S) and cruciferin (11S) proteins, both of which have distinct biological, physicochemical, and structural properties as well as industrial applications. This is distinct from other processes whose derived protein extracts are mixtures of 11S and 2S proteins. The aqueous extraction process provides various fractions of different physicochemical and functional properties that are expected to significantly enhance high-value application of Brassica proteins, beyond current traditional uses. A mass transfer study of a pilot plant setup was conducted together with an economic analysis of the extraction process. Simulations were conducted for capital equipment sizing and costs to predict various commercial-scale plant capacities. A full economic and financial analysis was completed including capital investment, annual manufacturing costs, primary and co-product revenues, product sales, risk-adjusted return on investment, and payback period, using an appropriate rate of return. The study shows that the economics of the aqueous extraction process are positive notwithstanding the fact that the aqueous extraction operating conditions had not yet been optimized.

Manufacturing New Food Co-Product(s) Using a Novel Ethanol Corn Fractionation System. T. T. Lohrmann, D.J. Hammes, Quality Technology International, Inc, Elgin, IL USA

A majority of the ethanol plants today are designed such that the entire corn kernel is ground prior to fermentation. The remaining non-fermentable fraction after fermentation is commonly dried and sold into the livestock markets as a lower value feed product termed distiller dried grains with solubles. The current focus of our technology is to convert ethanol plants from a traditional dry grind system into a next generation fractionation bio-refinery capable of making bulk food products such as high TDF corn bran and high purity corn germ for further processing to corn oil. Since our wet fractionation systems for ethanol do not utilize sulfur dioxide, production of high purity food products are attainable. Recent work by our group on second generation products has shown that corn germ proteins can be isolated and purified in to novel commercial food products. Other second generation, further-processed food products are in development which will bring even greater consumer benefits and value from this evolving industry.

Identification of Novel Co-product Opportunities From the Low Temperature Fermentation of Grains to Ethanol. D. Bressler, R. Zijlstra, A. Gibreel, University of Alberta, Edmonton, AB, Canada

With the support of the fermentation industry, research focused on determining the biofuel potential of several non-

traditional grains including barley, triticale, pulses and other crops with wheat and corn serving as benchmark feedstocks. Research into the fermentation of these non-traditional grains via both traditional jet-cooking and low temperature enzymatic approaches was conducted. An emphasis was placed on freeze-drying the resultant distillers grains and the final product were chemically profiled and assessed in terms of feed value as well as opportunities for the extraction of high value components. Chemical profiling clearly demonstrated that with newer low temperature enzyme approaches, several high-value components survived fermentation and were in fact concentrated three to five fold in the resulting solids fractions. The high-value components identified included tocopherol, tocotrienols, polyphenols, and phytosterols. Additionally, the fatty acid composition of the grains was preserved through fermentation. The identification of these potential co-products as an extraction opportunity prior to high temperature drying of distillers grains provides an opportunity for diversification of value-addition in the ethanol industry.

The Composition of Crude Corn Oil Recovered after Fermentation via Centrifugation from a Commercial Dry Grind Ethanol Process. R.A. Moreau[1], K.B. Hicks[1], D.B. Johnston[1], N.P. Laun[2], [1]ERRC, USDA, Wyndmoor, PA, USA, [2]Western New York Energy, Medina, NY, USA

A study was conducted to examine the chemical composition of corn oil obtained via centrifugation after fermentation of corn to make fuel ethanol, and compare its composition to that of corn germ oil (commercial corn oil) and experimental corn oils. The levels of free fatty acids in the post fermentation corn oil were high (13-16%), as previously reported. The levels of free phytosterols and hydroxycinnamate steryl esters (similar to oryzanol in rice bran oil) were higher than those of corn germ oil and were comparable to those of ethanol extracted corn kernel oil. The levels of tocopherols and tocotrienols were lower in post-fermentation oil than in either corn germ oil or ethanol extracted corn kernel oil. The levels of lutein and zeaxanthin in post-fermentation were much higher than those in corn germ oil and were comparable to those in ethanol extracted corn kernel oil. Overall, exposure to all upstream processes of a fuel ethanol plant, including high-temperature liquefaction, saccharification and fermentation appeared to have the greatest effect on tocopherols and tocotrienols, but it had little effect on the levels of free phytosterols, hydroxycinnamate steryl esters, lutein and zeaxanthin.

Changes in the Oil Concentration, Fatty Acid Composition, and Functional Lipid Profiles During Dry Grind Ethanol Production from Corn. Keshun Liu[1], Robert Moreau[2], Jill Moser[3], [1]USDA, ARS, Aberdeen, ID, USA, [2]USDA, ARS, ERRC, Wyndmoor, PA, USA, [3]USDA, ARS, NCAUR, Peoria, IL, USA

Demand for alternatives to fossil fuels has resulted in a dramatic increase in ethanol production from corn. The dry grind method has been the major process, resulting in a large volume of dried distiller grains with solubles (DDGS) as a co-product. This presentation reports our study to monitor concentration changes of various types of lipids during the entire dry grind process of corn. Samples of ground corn, intermediate products and DDGS were provided by three commercial plants in Iowa. After lyophilization, the moisture and crude oil levels as well as the fatty acid, phytosterol, tocopherol, and tocotrienol composition were measured. Results from Plant 1 samples show that the three steps that caused significant increases in oil content as compared with ground corn were slurrying, fermentation, and centrifugation (only into thin stillage). However, for all other lipids measured, including fatty acid composition (relative to total fatty acids), and composition of phytosterols, tocopherols and tocotrienols (relative to total lipid mass), the process did not cause significant changes, even at the three critical steps that caused significant increases in oil content. The conclusion is that the dry grind process caused a significant change in lipid quantities, but not lipid quality. Data from Plant 2 and 3 confirmed those found with Plant 1 samples.

Changes in Physical Properties During Dry Grind Processing of Corn. K.A. Rosentrater[1], K. Liu[2], [1]USDA, ARS, Brookings, SD, USA, [2]USDA, ARS, Aberdeen, ID, USA

Corn-based ethanol has dramatically increased in the U.S. in recent years. So too has the quantity of coproducts. These are composed of nonfermentable components (i.e., protein, lipid, fiber, and ash) from the corn kernel. These materials are separated from the ethanol and then subjected to various separations and drying processes. The most common coproduct is distillers dried grains with solubles (DDGS). DDGS has become widely used as a livestock feed ingredient. One of the key issues associated with DDGS is poor flowability. The objective of this study was to identify

where in the manufacturing process stickiness is imparted to the coproduct material. Eight samples were collected from two corn-based fuel ethanol plants, and included raw corn, cooked slurry, liquefied mash, whole stillage, thin stillage, wet cake, distillers dried grains (i.e., no added solubles), and DDGS. Each of these samples was dried at 50°C for 24 h, then milled to 0.5 mm. Properties tested included Carr Testing (angle of repose, aerated bulk density, packed bulk density, compressibility, angle of spatula, total flowability index, angle of fall, angle of difference, dispersibility, floodability index), thermal conductivity, thermal diffusivity, and color (Hunter L, a, b). Results of these tests will be presented and compared, and implications for the industry will be discussed.

Integrated Production of Ethanol and Succinic Acid in a Biorefinery. Nhuan P. Nghiem, Kevin Hicks, David Johnston, Eastern Regional Research Center USDA ARS, Wyndmoor, PA, USA

Production of succinic acid from glucose by Escherichia coli strain AFP184 was studied in a batch fermentor. The bases used for pH control included NaOH, KOH, NH4OH, and Na2CO3. The yield of succinic acid without and with carbon dioxide supplied by an adjacent ethanol fermentor using either corn or barley as feedstock was examined. The carbon dioxide gas from the ethanol fermentor was sparged directly into the liquid media in the succinic acid fermentor without any pre-treatment. Without the CO2 supplement, the highest succinic acid yield was observed with Na2CO3, followed by NH4OH, and lastly by the other two bases. When the CO2 produced in the ethanol fermentation was sparged into the media in the succinic acid fermentor, no improvement of succinic acid yield was observed with Na2CO3. However, several-fold increases in succinic acid yield were observed with the other bases, with NH4OH giving the highest yield increase. The yield of succinic acid with CO2 supplement from the ethanol fermentor when NH4OH was used for pH control was equal to that obtained when Na2CO3 was used, with or without CO2 supplementation. The benefit of sparging CO2 from ethanol fermentation on the yield of succinic acid demonstrated the feasibility of integration of succinic acid fermentation with ethanol fermentation in a biorefinery.

Assessment of a High Purity Zein Product from Commercial Zein. David J. Sessa, Kristen Kruger Woods, Plant Polymer Research, NCAUR, USDA, ARS, Peoria, IL, USA

Successful utilization of commercial zein products for food, pharmaceutical, cosmetic and medical applications requires a decolorized/deodorized zein that is substantially undenatured protein. A zein protein with those qualifications has already been developed by a patent pending process. The objective of this presentation is to devise methodologies to assess the required attributes. Zein purity was assessed by FTIR and circular dichroism (CD) of commercial zein before and after further purification. Spectral differences were observed in the amide I (1650 cm^1^) peak, amide II region (1530 and 1515 cm^1^) and the amide III peak at 1240 cm^1^, where ratios of dominant peaks were strongly dependent on purity of sample. CD analyses validated FTIR results by showing increased α-helical content for purified zein. We defined off-odor removal by UV spectroscopic ratio of 280nm:325nm of ≥8, where diferoylputrescine is the major contributor. Color removal, attributed to xanthophylls in zein was followed by a visible spectroscopic assay. These results are a major contribution for achieving a consistent zein product.

BIO 1.1: Biocatalysis I
Chair(s): C. Hou, Renewable Product Technology Research Unit, NCAUR, ARS, USDA, USA; and J. Ogawa, Kyoto University, Japan

A Novel Aliphatic Amino Acid Metabolism in Bacteria Generating a Potential Insulinotropic and Anti-obesity Amino Acid. J. Ogawa[1,2], T. Kodera[3], S. V. Smirnov[4], N. N. Samsonova[4], M. Hibi[5], K. Yokozeki[5,6], S. Shimizu[1], [1]Division Applied Life Science, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Res. Div. Microb. Sci., Kyoto University, Kyoto, Japan, [3]Inst. Life Science., Ajinomoto Co., Inc., Kawasaki, Japan, [4]Ajinomoto-Genetika Res. Inst., Moscow, Russia, [5]Lab. Ind. Microbiol., Grad. Sch. Agric., Kyoto Univ., Kyoto Japan, [6]Aminosci. Lab., Ajinomoto Co., Inc., Kawasaki Japan

4-Hydroxyisoleucine (HIL) is a polar non-charged amino acid and was found to be an active component of fenugreek seeds, which are used in spices and in traditional medicines as a remedy for diabetes and hypercholesterolemia. Based on its insulinotropic and anti-obesity effects, HIL might be considered as a novel orally-active drug with potential for

the treatment of insulin-independent diabetes mellitus and obesity. Here we show that newly isolated Bacillus thuringiensis strain 2e2 operates a novel metabolic pathway for L-isoleucine via 4-hydroxyisoleucine (HIL) and 2-amino-3-methyl-4-ketopentanoic acid (AMKP). The HIL synthesis was found to be catalyzed stereoselectively by a novel alpha-ketoglutaric acid-dependent (succinic acid-producing) dioxygenase and to be useful for efficient production of a naturally occurring HIL isomer, (2S,3R,4S)-HIL. The metabolic pathway functions as an effective by-pass pathway that compensates for the incomplete TCA cycle in B. thuringiensis, which lack the alpha-ketoglutaric acid dehydrogenating activity being necessary to generate succinic acid, and also explains how AMKP, a vitamin B12 antimetabolite with antibiotic activity, is synthesized. These novel findings pave a new way for commercial production of HIL and also for AMKP.

Chiral Technologies for Single Enantiomer Drug Intermediates Through Biocatalysis. Ramesh N. Patel[1,2], Sandip J. Parekh[2], [1]SLRP Associates, Bridgewater, NJ USA, [2]Unimark Remedies, Ltd, Mumbai, India

Biocatalytic processes for producing chirally pure pharmaceutical intermediates are of growing importance. Biocatalysis is rapidly evolving into a key technology for the production of fine chemicals and chiral intermediates where high yielding chemo-, regio-, and enantioselective reactions are critical. Advances fermentation technology and development in molecular biology methods such as over expression of enzymes and directed evolution technologies have now lead to improvement in activity, selectivity and stability of biocatalyst under process conditions. Biocatalysis provides advantage offering higher yields, fewer side reactions, elimination of protection and de-protection steps and reduced environmental waste. Operational advantages, including the ability to carry out reactions under mild operational conditions, avoiding extremes of pH, temperature, and pressure that often require the use of expensive equipment or energy intensive processing. Furthermore this minimizes problems of undesired side-reactions such as decomposition, isomerization, racemization and rearrangement which often plague traditional methodology. In this presentation various biocatalytic processes for the preparation of chiral intermediates for drugs will be described.

Characterization of Milkweed Oil and Cis-vaccenate Biosynthesis. Thomas McKeon, Frank Rittig, Charlotta Turner, USDA, ARS, WRRC, Albany, CA 94710, USA

We evaluated the fatty acid composition in seeds of 22 Asclepias (milkweed) species, using supercritical fluid extraction to obtain the seed oil. We then compared transesterification methods, using methanolic HCl and lipase catalyzed transesterification in supercritical CO2. We found that under the conditions we used, the lower pressure of 470 bar was effective for quantitative extraction of seed oil from Asclepias, and that addition of methanol as a modifier was unnecessary. Results obtained for the two transesterification methods indicate that both were generally in close agreement, with some differences observed for vaccinate and linoleate content. Because milkweeds contain an unusual amount of cis-vaccenate in its oil, we compared the ability of developing milkweed (A. currasavica) seeds to desaturate palmitoyl-ACP and stearoyl-ACP seeds. The ratio of stearate to palmitate desaturation was 4.1. These results are consistent with the vaccenate arising from desaturation of palmitoyl-ACP and elongation of the palmitoleate to cis-vaccenate.

Construction and Characterization of Fusion Class III Poly(hydroxyalkanoate) Biopolymer Synthase Genes. D.K.Y. Solaiman, R.D. Ashby, J. Zerkowski, ERRC, ARS, USDA, Wyndmoor, PA, USA

Poly(hydroxyalkanoates) (PHAs) are polyesters produced by bacteria. Because these polymers arebiodegradable and produced from renewable resources, PHAs are studiedas environmentally friendly replacement for petroleum-based polymers. High production costsand limited applications impede the widespread adoption of PHAs, however.Genetic engineering of PHA-producing bacteria can help overcome this problem.Poly(hydroxyalkanoate) synthase (PHAS) is an enzyme responsible for PHA synthesis. We will discuss in this talk our study to genetically fuse the genes encoding thetwo subunits of a PHAS of Allochromatium vinosum, i.e., the phaE and phaC genes. Wehypothesize that the fused genes will facilitate the association of the two subunits toincrease product yield. Two fused genes (phaEC and phaCE) are constructed using theoverlapping-primer-extension PCR method. The overlapping primers were designed in such a waythat a stretch of 6 amino-acid sequence links the two fused pha genes in the resultant phaECand phaCE genes. The progress of the ongoing experiments to express the fused genes inPseudomonas resinovorans and Ralstonia eutropha using an expression vector, pBS29-P2, will bediscussed in detail in the presentation. The results should yield important

information forimproving production yield of PHA in genetically modified organisms.

Screening of Microbial *n*-Alkane Degradation through Subterminal Oxidation. Eiji Sakuradani, Koji Kobayashi, Nozomu Shibata, Jun Ogawa, Sakayu Shimizu, Kyoto University, Kyoto, Japan

The terminal oxidation is well-known as a microbial oxidation of *n*-alkanes. Thus far, the genes and their enzymes related with the terminal oxidation have been studied in detail. However, the studied related with the microbial sub-terminal oxidation are hardly progressed. Previously, we reported that an achlorophyllous micro-alga, *Prototheca zopfii*, oxidizes 5th carbon of *n*-hexadecane through the sub-terminal oxidation to generate 5-hexadecanol and 5-hexadecanone. We have screened various microorganisms with the sub-terminal oxidation of *n*-hexadecane, and found that actinomycete *Streptomyces griseus*, basidiomycete *Schizophyllum* sp., and ascomycete *Penicillium janthinellum* oxidize mainly 4th, 6th, and 9th position of *n*-hexadecane, respectively, through the sub-terminal oxidation pathway. Characterization of the sub-terminal oxidation pathway in the microorganisms obtained through the screening will be discussed.

Production of Biologically Active 7, 10-dihydroxy-8(E)-octadecenoic Acid from Korean Pine Seed Oil by Pseudomonas aeruginosa PR3. H.R. Kim[1], K.Y. Baek[1], J.B. Ellamar[1], C.T. Hou[2], M.H. Kwon[1], H.R. Lim[1], [1]Department of Animal Science and Biotechnology, Kyungpook National University, Daegu, Korea, [2]Microbial Genomic and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL USA

Hydroxyl group of hydroxyl fatty acid (HFA) cause HFA to carry special properties such as higher viscosity and reactivity compared with other non-hydroxy fatty acids. Microbial conversion of oleic acid into 7,10-dihydroxy-8(E)-octadecenoic acid (DOD) caused DOD to carry strong antibacterial activity against several food pathogenic bacteria. However, industrial application of DOD as an antibacterial agent is limited by its high production cost. Hence we tried to use vegetable oil as a substrate for DOD production by Pseudomonas aeruginosa (PR3). Of those vegetable oils tested, we selected Korean pine seed oil as a substrate and studied about optimal culture conditions for DOD production.

Metabolism and Synthesis of Lipids in Polyunsaturated Fatty Acid-producing Fungus, *Mortierella*. T. Aki, W. Jermsuntiea, S. Kawamoto, K. Ono, Hiroshima University, Higashi-Hiroshima, Japan

Linoleic Acid Isomerase in *Lactobacillus plantarum* AKU1009a is a Multi-component Enzyme System Requiring Oxidoreduction Cofactors. Shigenobu Kishino[1,2], Si-Bum Park[1], Yuki Ishigaki[1,2], Jun Ogawa[2,3], Kenzo Yokozeki[1], Sakayu Shimizu[2], [1]Laboratory of Industrial Microbiologys, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan, [2]Division of Applied Life Sciences Graduate School of Agriculture, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan, [3]Research Division of Microbial Sciences, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

Conjugated linoleic acid (CLA) is a collective term for positional and geometric isomers of linoleic acid (LA) with conjugated double bonds. CLA has potentially beneficial effects, for example, prevention of carcinogenesis, and reduction of body fat content. Natural sources of CLA are daily products from ruminants, although their CLA contents are very low. We screened the ability to produce CLA from LA within lactic acid bacteria, and selected *Lactobacillus plantarum* AKU 1009a as a potential strain. This strain produced two CLA isomers, i.e., *cis*-9,*trans*-11 and *trans*-9,*trans*-11-CLA, from LA with 10-hydroxy-12-octadecenoic acids (18:1) as intermediates. These results suggested that the reaction involves hydration and dehydrating isomerization catalyzed by multiple enzymes. Then we clarified three enzymes (CLA-HY, CLA-DH, and CLA-DC) from *L. plantarum* AKU 1009a involved in CLA production from LA. CLA-HY was a membrane protein and catalyzed hydration reaction. LA was hydrated to 10-hydroxy-12-18:1 by CLA-HY. Further transformation of 10-hydroxy-12-18:1 to CLA and LA transformation to CLA were only detected in the presence of all three enzymes, i.e., CLA-HY, CLA-DH, and CLA-DC, and the multi-component system

required NADH and FAD as the cofactors for its maximal activity.

Hydroxyl Fatty Acids and Hydroxyl Oils. C.T. Hou, Renewable Product Technology Research Unit, NCAUR, ARS, USDA, Peoria, IL 61604, USA

Soybean oil is produced domestically in large supply, averaging over 20 billion pounds per year with an annual carryover of more than one billion pounds. It is important to find new uses for this surplus soybean oil. Hydroxyl fatty acids and hydroxyl oils are platform materials for specialty chemicals and oil industry. Many years ago we started a research project to convert soybean oil and its component fatty acids to value-added products such as recinoleic acid. We successfully found many microbes that can convert fatty acids to recinoleic acid-type hydroxyl fatty acids. We also discovered many new bioactive or potentially bioactive fatty acids. Now we need a direct bioprocess to convert soybean oil to hydroxyl fatty acids. Soy polyols (hydroxyl triacylglycerols) are important starting materials for the manufacture of polymers such as polyurethane. Currently, there are two chemical processes to produce triacylglyceride polyols, and these involve two steps: epoxidation and then opening of oxidane ring. It is important to have a non-polluting bioprocess that can directly convert soybean oil to polyol oils. Following our previous experience, we should be able to find microorganisms that can convert soybean oil directly to soy polyol oils.

AFTERNOON

BIO 1.2 / PCP 1: Process and Co-products of Biofuel and Industrial Production

Chair(s): K. Liu, USDA, ARS, PWA, USA; D. Solaiman, USDA, ARS, ERRC, USA; J. Wanasundara, Agriculture & Agri-Food Canada, Canada; and H. Wang, Iowa State University, USA

Proteins as Renewable Flocculants. G. Piazza, R. Garcia, ERRC, ARS, USDA, Wyndmoor, PA, USA

Nine billion chickens are slaughtered in the U.S. per year, but the protein rich chicken blood (CKB) is mainly treated as waste. We have investigated the use of CKB fractions as replacements for a widely used nonrenewable, polymeric flocculant, anionic polyacrylamide (PAM), which requires the addition of calcium chloride for activity. PAM is needed for several municipal and industrial operations. The CKB frozen fractions studied were (A) blood, freed of coagulated blood; (B) blood plasma (blood centrifuged to remove blood cells); and (C) heated blood, centrifuged to remove solids. These CKB fractions were dehydrated two ways: freeze drying and spray drying at elevated temperature. Flocculation activity was quantified by determining the concentration of flocculant needed to settle a constant amount of suspended clay particles in 1 h, 5 h and 24 h. Flocculation activity by the CKB fractions required the addition of buffer, citric acid, phosphoric acid or sulfuric acid to reduce the pH to 5.5, but does not require the addition of calcium chloride. The flocculation activity of frozen and freeze dried CKB fractions was superior or equivalent to that of anionic PAM. The flocculation activity of spray dried fractions A and B was nearly as good as that of anionic PAM. The results indicate that CKB fractions may be viable renewable replacements for PAM.

BIO 2: Sterols II

Chair(s): G.I. Lepesheva, Vanderbilt University, USA; and W.D. Nes, Texas Technical University, USA

Identification of Natural Ligands for the Nuclear Hormone Receptor RORγ. Fabio R. Santori[1], Varun Sondhi[2], Brittany Rosales[3], H. Eric Xu[4], W. David Nes[3], David Mangelsdorf[2], Dan R. Littman[1], [1]Skirball Institute Of Biomolecular Medicine, NYU School of Medicine, New York, New York, USA, [2]Howard Hughes Medical Institute and Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA, [3]Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas, USA, [4]Laboratory of Structural Sciences, Van Andel Research Institute, Grand Rapids, Michigan, USA

RORg is a transcription factor of the Nuclear Hormone Receptor (NHR) Superfamily. Ligands for NHRs have been identified and generally consist of small lipophilic molecules. Although, great progress has been achieved in the last years in the identification of natural ligands for NHRs, nearly half of the known human NHRs are orphan receptors. RORg plays an important role in development of lymphoid tissues like thymus, lymph nodes, gut associated lymphoid tissue (GALT) and pro-inflammatory Th17 cells.The important role played by ROR in the development and

physiology of the immune system has increased the interest on the identification of natural ligands that regulate RORg activity â??in vivoâ?. The identification of such molecules would allow the study of cellular mechanisms and pathways regulated by RORg that are required for the differentiation of Lti, Lti-like and Th17 lineages. To that end, we have designed luciferase based reporter systems as sensors for compounds that can induce RORg activity. Moreover, chemically defined synthetic media has allowed us to test the requirement of several endogenous lipids for RORg reporter activity. These experiments, supported by RNAi inhibition of lipid biosynthetic enzymes in mammalian cells have suggested that RORg ligands maybe derivatives of sterol lipids.

Genetic Dissection of AACT Paralog-function on the Phytosterol Profile of Arabidopsis. Zhihong Song[1,2], Huanan Jin[2], Basil J. Nikolau[1,2], [1]The Ames Laboratory of US DOE, Ames, IA, USA, [2]Iowa State University, Ames, IA, USA

Acetoacetyl-CoA thiolase (AACT) catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. In the cytosol of plant cells, it is the precursor of mevalonate-derived isoprenoids. Two AACT genes, At5g47720 (AACT1) and At5g48230 (AACT2), were identified in the Arabidopsis genome by BLASTP analysis. Two T-DNA mutants at each AACT gene were characterized. These characterizations indicate that although both genes are expressed, mutations in AACT2 are embryo lethal whereas null alleles of AACT1 are viable and show no apparent growth phenotypes. Promoter::GUS fusion experiments indicate that AACT1 is primarily expressed in the vascular system and AACT2 is highly expressed in root tips, leaves, stems and anthers. AACT2 RNAi knock-down lines show pleiotropic phenotypes, including dwarfing, reduced root length, early senescence, elongated flowering time, sterility, reduced cell size, early degradation of tapetum cells and loss of pollen coat. These phenotypes are rescued when the plants are grown in the presence of mevalonate. Phytosterol analysis of AACT2 RNAi lines show reduced accumulation of sitosterol, stigmasterol, campesterol, and all other sterols in the root. The accumulation of these sterols is increased to wild type levels by the application of exogenous mevalonate. In contrast, no significant phytosterol changes are detected in the aact1 mutant.

Bioengineering Strategies for Enhanced Phytosterol Accumulation in Soybean Seeds. Anjanasree Neelakandan[1], Swetha Chamala[2], Babu Valliyodan[1], W. David Nes[2], Henry Nguyen[1], [1]University of Missouri, Columbia, MO, USA, [2]Texas Tech University, Lubbock, TX, USA

Plant sterols are an important class of nutritional and pharmaceutical biomolecules found only in minor proportions in commercial seed oils, including soybean, which are the major dietary sources. In an attempt to generate value added soybeans enriched in cholesterol lowering 4-desmethylsterols, we over-expressed soybean Sterol Methyltransferase1 (SMT1) gene, catalytic subunit of HMGR1 gene from Arabidopsis and Sterol Methyltransferase2-2 (SMT2-2) gene from soybean. We found only modest increase in total sterol levels (3-6%), which points towards a tight control of this trait in soybean seeds. However, beneficial alterations in sterol composition as evidenced by higher end product sterol accumulation (90% of total sterols, as against 65% in wild type) were noted in plants containing seed-specific promoters, whereas increased accumulation of intermediate sterols (55% of the total as opposed to 35% in wild type) was observed with constitutive HMGR1 over-expression. The intermediates abundantly present in soybean seeds are efficiently converted to sterol end-products which are important for cholesterol lowering and other therapeutic effects. Taken together, our results demonstrate the usefulness of transgenic technology for obtaining desirable sterol composition in target tissues in important oilseed crops like soybean.

Phytosterols as Novel Tools for Controlling Insect Pests. S. Behmer[1], R. Grebenok[2], A. Douglas[3], [1]Texas A&M University, College Station, TX, USA, [2]Canisius College, Buffalo, NY, USA, [3]Cornell University, Ithaca, NY, USA

Exploiting nutritional requirements unique to insects offers a novel, target-specific and environmentally friendly approach for controlling insects that attack agricultural crops. A key nutritional requirement for insects is the need to acquire a dietary source of sterol, which is, among other things, used in cell membranes and as a precursor to molting hormone. Cholesterol is the most abundant sterol found in plant-feeding insects, but it is rare in plants, so plant-feeding insects generate cholesterol by metabolizing ingested plant sterols. There are, however, plant sterols that plant-feeding insects cannot convert to cholesterol, and the introduction of these ?bad? sterols into crop-plants might provide a novel

form of plant-defense. This talk explores how two groups of plant-feeding insects (caterpillars and aphids) are affected by changes in the sterol profile of their food. This question is investigated using tobacco plants with modified sterol profiles, and artificial diets with known sterol profiles. The results from this work reveal interesting outcomes, both in terms of plant and insect physiology, and suggest that the development of transgenic crops, containing modified sterols, has real potential for managing insect crop pests.

Structural Insights into Selective Inhibition of Protozoan Sterol 14alpha-demethylase. G.I. Lepesheva, Vanderbilt University, Nashville, TN, USA

Human infections with protozoan pathogens such as trypanosomes and leishmania have been threatening lives of millions of people for hundreds of years. With human migrations and broadening of the host reservoir due to climate changes, they are now spreading all over the world as a result of HIV-coinfections, blood transfusions, organ transplantation, food contamination, and yet their treatment remains unsatisfactory. Broad search for new drug targets based on the Trypanosomatidae biology proves that sterol biosynthesis has a high potential as a drug target pathway, sterol 14alpha-demethylase being one of the most promising drug targetable enzymes. Crystal structures of sterol 14alpha-demethylases from three protozoan pathogens, Trypanosoma brucei, Trypanosoma cruzi and Leishmania infantum, ligand-free and complexed with different inhibitors, solved recently in our laboratory, provide a molecular basis for better understanding the enzyme inhibition, functional conservation across the biological kingdoms, explain some cases of drug resistance, and open an excellent opportunity for rational, structure-based drug design.

New Methods for Oxysterol Synthesis: Allylic Oxidation of Steroids and Sterols. E.J. Parish, A.D. Bell, D. Lu, A.E.V. Gorden, Y. Li, X. Wu, T. Lee, Department of Chemistry and Biochemistry, Auburn University, AL, USA

Allylic oxidation of steroids, particularly at the 7-position, has attracted interest over many years. The 5-ene steroids can be oxidized to the 5-en-7-one, which are known as inhibitors of sterol biosynthesis and cell replication. They also have use in cancer chemotherapy, since they are more toxic towards tumorous than non-tumorous cells. We have recently developed two new reagents which have proven to be useful for the allylic oxidation of unsaturated steroids and sterols (steroidal alkenes) to the corresponding unsaturated ketosteroids. These are Magtrieve (chromium dioxide) and a tert-butylhydroperoxide oxidant with a modified salen-quinoxalinol Cu(II) complex catalyst. Both of these reagents have been able to replace chromium salts and chromic acid for this purpose and eliminate toxic wastes. The latter method is able to directly preform allylic oxidation of sterols to ketosterols without oxidation of the alcohol (hydroxyl) functional group of sterols.

BIO 2.1 / PRO 2.1: Processing Technologies
Chair(s): X. Xu, University of Aarhus, Denmark; and N. Dunford, Oklahoma State University, USA

Advantages and Challenges in Enzymatic Catalyzed Biodiesel Production. P.M. Nielsen, J. Brask, H. Lilbaek, M.L. Damstrup, A.R. Madsen, H.C. Holm, Novozymes, Bagsvaerd, Denmark

It is well-known that the choice of alcohol is an important factor for determining enzyme stability in a transesterification reaction. However, it further appears that enzymatic production of fatty acid ethyl ester, relative to conventional production of methyl esters, can be beneficial for both process economy, as well as from a sustainability viewpoint. The different aspects of the alcohol choice will be discussed, and the consequences of using different oil types and qualities will be illustrated.The process plant layout itself is very important for the lifetime of the enzyme and thereby the economy. A separate question is related to the enzyme product. Not only the enzyme catalyst itself but also the way it is formulated is crucial for the performance. We will discuss results from using different types of lipase, different carriers for immobilizing the enzyme, as well as the possibility of using liquid enzymes.There is not a one-fits-all solution for the enzymatic process. The enzymatic production of biodiesel will be an important opportunity for some markets with the possibility of expansion from the technology base we seek to establish for ethyl ester production into general application of enzymes with different types of oil raw materials.

Evaluation of Reaction Engineering Parameters in Enzyme-based FAEE-biodiesel Processes. M. Nordblad, Y. Xu, J.M. Woodley, Process Engineering and Technology Group, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark

Biodiesel is receiving increasing attention as a promising alternative to liquid fossil fuel for vehicles, being a direct replacement for petrochemical diesel. Compared to the conventional biodiesel production process, enzymatic synthesis offers several advantages such as lower reaction temperature, eliminated soap formation and simplified product recovery. Unfortunately, the cost of the enzyme-based catalysts is considerably higher than that of the alkaline catalysts used in the conventional process. It is thus critical that the enzyme-based catalysts can be reused.The reaction conditions greatly affect the performance of enzymatic biodiesel production. For example, the concentration of alcohol in the system affects both the reaction rate and the catalyst stability. In order to maximize the amount of biodiesel that can be produced by a given amount of catalyst, it is useful to design the process to maintain controlled substrate levels. However, the production of biodiesel is a challenging process in itself, with very strict requirements on product quality for what is essentially a low-value product, and it is essential to consider this alongside the choice of operating parameters. This study presents an evaluation of the options available for the enzymatic production of biodiesel and the consequence of these for the overall design and control of the process.

Development of Reactor Technology for Improved Catalytic Productivity in Enzymatic FAEE-biodiesel Production. Y. Xu, M. Nordblad, J.M. Woodley, Process Engineering and Technology Group, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark

Biodiesel production catalyzed by immobilized lipases is attracting considerable interest as an alternative to conventional base-catalysed biodiesel production due to its potential of improved sustainability[1]. However, the high cost of the immobilized lipases has limited commercial application. High process efficiency could be the key to achieving high productivities of the expensive immobilized lipases and improved economic viability for large industrial application; the reactor is the core of an efficient process design. This work has taken the stirred tank reactor and the packed bed reactor as examples of reactor technology development in order to improve the productivities of the immobilized lipases for FAEE-biodiesel production.One of the major factors causing insufficient stability of immobilized lipases is glycerol, the major by-product of biodiesel production[2]. A dyeing method for indicating glycerol partitioning has been developed and applied in stirred tank and packed bed reactors to assist the reactor evaluation and its effect on catalyst productivity. The mechanical stability of the immobilized lipases has also been evaluated in these reactors. [1] Nielsen, P.M. et al., 2008. Eur. J. Lipid Sci. Technol., 110, 692. [2] Dossat, V. et al., 1999. Enzyme Microb. Technol., 25, 194.

Enzymatic Biodiesel, Monitoring and Analysis of the Reaction. Sergey N. Fedosov, Xuebing Xu, Agrobiology Group, Dept. Molecular Biology, Århus University, Århus, Denmark

Biodiesel (BD) is produced by treatment of vegetable oil with alcohol in the presence of a catalyst. Application of the enzyme lipase for this purpose is presently debated, because the enzyme has low sensitivity to oil composition and produces minimum waste. Optimization of the complex reaction kinetics is, however, required. Multiple measurements make the well established analytical methods of gas-chromatography, HPLC and TLC-FID inconvenient in terms of time, labor and expenses. The current work describes two suitable analytical approaches. First of them is a low-cost method, where the major components of reaction (BD, tri/di/mono-glycerides, fatty acids) are separated on a standard TLC plate and stained. Absence of background in the suggested procedure simplifies quantification by densitometry or visual assessment. The second method is based on measurements of fluorescent signal from a probe sensitive to polarity of the environment. This approach is useful when investigating partial reactions accompanied by a change of polarity (e.g. MG + MeOH → BD + G). Fluorescent method requires calibration of the signal and is convenient if repeated measurements are carried out. Examples of the calibration curves and the reaction records are presented. Analysis of partial reactions is discussed as an approach to simplify reconstruction of the global scheme of BD-synthesis.

Predictive Studies on Phase Equilibria of Enzymatic Biodiesel Production. G. Güzel, X. Xu, Aarhus University, Department of Molecular Biology, Aarhus, Denmark

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Synthesis of Diacylglycerols: Lipases vs. Macroreticular Strongly Acidic Cation Exchange Resins. O.M. Lai[1,2], S.K. Lo[3], [1]Universiti Putra Malaysia, Serdang, Selangor, Malaysia, [2]Institute of Bioscience, Serdang, Selangor, Malaysia, [3]Sime Darby Research Centre, Banting, Selangor, Malaysia

Much has been published about the use of lipases for the production of structured lipids such as diacylglycerols. We now report of a chemical catalyst for the synthesis of of dietary 1,3(2)-diacylglycerol. Lipases and macroreticular strongly acidic cation exchange resin were used to catalyze the esterification of stearic and oleic acids with glycerol to synthesize 1-stearoyl-3(2)-oleoyl glycerol. A dual response surface approach was used to optimize both the esterification reaction variables and the optimized conditions for both the catalysts were compared and reported.

Enzyme-Catalyzed Production of Structured Lipids under High-Pressure Conditions. S. Ferreira-Dias[1], N.M. Osório[2,1], C. Tecelão[3,1], V. Perrier[4], E. Dubreucq[4], M.H. Ribeiro[5], [1]Instituto Superior de Agronomia, Technical University of Lisbon, CEER, Lisbon, Portugal, [2]Instituto Piaget, Núcleo de Investigação em Engª Alimentar e Biotecnologia, ISEIT de Almada, 2800-305 Almada, Portugal, [3]Escola Superior de Turismo e Tecnologia do Mar, Instituto Politécnico de Leiria, 2520-641 Peniche, Portugal, [4]Montpellier SupAgro, UMR 1208 IATE, F-34060 Montpellier cedex, France, [5]Faculdade de Farmácia, Research Institute for Medicines and Pharmaceutical Sciences (i-Med.UL), University of Lisbon, 1649-003 Lisbon, Portugal

TUESDAY

AFTERNOON
BIO 3: Plant Lipid Biotechnology
Chair(s): R. Weselake, University of Alberta, Canada; and R.F. Wilson, Oilseeds and Biosciences Consulting, USA

Biogenesis of ER Subdomains Containing DGAT2, an Enzyme Involved in Industrial Oil Biosynthesis. S.K. Gidda[1], J.M. Shockey[2], R.T. Mullen[1], J.M. Dyer[3], [1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada, [2]USDA-ARS, Southern Regional Research Center, New Orleans, New Orleans, LA, USA, [3]USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ, USA

Diacylglycerol acyltransferases (DGATs) are enzymes that catalyze the committed step in triacylglycerol (TAG) biosynthesis by transferring a fatty acyl group from the acyl-CoA pool to the sn-3 position of diacylglycerol. The substrate specificity and overall activity of these enzymes play a key role in determining the quantitative and qualitative properties of seed storage oils. Plants contain two unrelated types of DGAT enzyme called DGAT1 and DGAT2, and we have recently shown that these enzymes have different substrate preferences, that they are located in different regions or ?subdomains? of the endoplasmic reticulum (ER), and that DGAT2 plays an important role in the channeling of either conjugated fatty acids or hydroxyl fatty acids into storage oils. The knowledge of DGAT2 subdomain biogenesis, therefore, will likely increase our ability to rationally engineer oilseed crops to produce industrially important oils. Here we describe the identification of two glycerol-3-phosphate acyltransferases (GPATs) from tung (Vernicia fordii) and demonstrate that both proteins target to the same subdomain of ER that is shared by DGAT2. Using a combination of mutational analyses and protein-protein interaction assays, we demonstrate that the GPAT's first transmembrane-spanning domain, which contains a leucine-zipper-like motif, is responsible for targeting to ER subdomains. These results demonstrate that the enrichment of lipid-biosynthetic enzymes into specific regions of the ER is dependent in part on formation of higher-ordered, oligomeric structures via protein-protein interactions. Implications for oilseed engineering are discussed.

FADX, DGAT, CPT, PDAT and More: a Vegetable Soup of Enzymes that Influence Industrial Oil Production in Transgenic Oilseeds. J.M. Shockey, USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA

Our laboratories study tung tree (Vernicia fordii) and bitter gourd (Momordica charantia) as model systems for the production of industrially useful oils. Seed oils of bitter gourd and tung contain approximately 60% and 80%, respectively, α?eleostearic acid, a valuable conjugated fatty acid. Successful reconstitution of the biosynthetic pathway for drying oils in agronomically amenable transgenic systems may require introduction of several genes. Some important components of these pathways, such as the fatty acid conjugases (FADXs) and diacylglycerol acyltransferases (DGATs) have been identified. We are currently focused on the optimization of the FADX and DGAT transgenes currently in use. The effects of alterations in transgene construct design, including changes that affect transcriptional timing and maintenance, translational efficiency, and protein stability will be presented. Other necessary pieces of the TAG biosynthetic puzzle remain elusive. Much effort has been focused on finding other enzymes in the pathway that contribute to efficient channeling of eleostearate into TAG. Recent results from the study of co-expression of genes located in the central part of the pathway (between FADX and DGAT) will also be presented.

Producing DHA in Canola Oil Via Algal PUFA Synthases. T. Walsh[1], J. Metz[2], [1]Dow AgroSciences, Indianapolis, IN, USA, [2]Martek BioSciences, Boulder, CO, USA

The unique human health benefits of the omega-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA) are well established and continue to be expanded. With increasing demand for convenient, low-cost sources of DHA, plant oils can potentially offer a healthy and sustainable method of production of this important omega-3 fatty acid. Canola (Brassica napus) is particularly suited as a production vehicle for DHA-containing plant oils because of its convenient scale, established grain identity-preservation channels and healthy oil attributes. In parallel, the use of genes encoding algal PUFA synthases offer a new and simplified mechanism for DHA synthesis in crop plants, and an alternative to strategies involving multiple elongation/desaturation steps of native fatty acids. Progress toward engineering canola oil to contain DHA using algal PUFA synthase genes will be described.

Enhancing Carotenoid Production in *Brassica napus* Seeds. Abdelali Hannoufa, Agriculture and Agri-Food Canada, London, Ontario, Canada

The accumulation of carotenoids in B. napus seed varies during development with beta-carotene and lutein accounting for more than 90% of the total content. The highest levels are detected at