MONDAY

AFTERNOON

BIO 1: Biocatalysis I

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

Microbial Oxygenases as Catalysts for Fine Chemical Synthesis. J. Ogawa[1], M. Hibi[2], K. Yokozeki[2], S. Shimizu[1,3], [1]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Sakyo–ku, Kyoto, Japan, [2]Lab. Ind. Microbiol., Grad. Sch. Agric., Kyoto Univ, Sakyo–ku, Kyoto, Japan, [3]Fac. Bio-environ. Sci., Kyoto Gakuen Univ., Kameoka, Kyoto, Japan

α-Ketoglutarate-dependent dioxygenases are useful for the production of hydroxy amino acids, some of which exert worthwhile physiological activities on the mammals, and also are components of peptide antibiotics and chiral precursors in the chemical synthesis of other compounds. We developed practical bioprocess system of (2S,3R,4S)–4–hydroxyisoleucine, which had remarkable anti-diabetic activity, by using an α–ketoglutarate dependent dioxygenase (IDO) derived from Bacillus thuringiensis 2e2 as a biocatalyst. We analyzed catalytic characteristics of several novel dioxygenases as well as IDO, toward developing effective production processes of various hydroxy amino acids. Then it was found that IDO and two dioxygenases, derived from Nostoc punctiforme and Burkholderia ambifaria, were good biocatalysts for production of various amino acid derivatives. Some of 4–hydroxy, 5–hydroxy, and 3–hydroxy amino acids, and also several 3–hydroxy N–substituted amino acids, and interestingly amino acid sulfoxides, were produced highly stereospecifically.

Microbial Screening and Analytical Methods for the Production of Polyol Oils from Soybean Oil. Ching T. Hou, Karen Ray, NCAUR, ARS, USDA, Peoria, Illinois, USA

The objective of this study is to develop a new useful method including microbial screening and product identification for a bioprocess to produce polyol oils from soybean oil. Methods for separating of product polyol oils from substrate soybean oil and byproducts free fatty acids using HPLC and TLC were established. With HPLC, a C18 reverse phase column and a gradient of 100% methanol to 100% 2–propanol over 60 minutes produced the best result. Free fatty acids were eluted at between 4.5 min to 11 min. Products polyol oils were eluted at between 15 min to 26 min. And the substrate soybean oil was eluted at between 35 min to 44 min. A TLC with two step development solvent systems was also developed to separate polyol oil products from substrate. A total of 100 microbial cultures were screened and found 25 hits. Among these 25 hits, Acinetobacter sp. A01–35 was the best strain for the production of polyol oil from soybean oil. Polyol oils products were purified and their chemical structures confirmed by HPLC/MS. This is a useful microbial screening and analytical methods for the production of polyols oils directly from soybean oil.

(R)–3–hydroxyacyl–ACP:CoA transacylase of Pseudomonas Chlororaphis: Gene Cloning, Characterization and Knock–out on PHA and Rhamnolipid Syntheses. D.K.Y. Solaiman, R.D. Ashby, J.A. Zerkowski, U.S. Department of Agriculture, ARS, ERRC, Wyndmoor, Pennsylvania 19038, USA

Pseudomonas chlororaphis is a useful microorganism capable of producing polyhydroxyalkanoate (PHA) biopolymer and rhamnolipid (RL) biosurfactants by using carbon– and nitrogen–sources derived from renewable feedstocks as substrates of fermentation. We are interested in increasing the yield of RL production to economize the process. The biosynthesis of PHA and RL both require 3–hydroxyacyl molecule as a precursor. (R)–3–hydroxyacyl-ACP:CoA

transacylase (PhaG) is the enzyme responsible for diverting the flow of 3–hydroxyacyl precursor to the PHA biosynthesis pathway. We hypothesize that by inactivating the PhaG activity, the precursor flow could be directed to favor RL production. In this presentation, we describe the cloning and characterization of the P. chlororaphis phaG gene, and the subsequent construction of phaG–negative knock–out P. chlororaphis mutants. These mutants are important for testing the effects of phaG inactivation on PHA and RL biosynthesis, which could lead to an increased and economic production of RL biosurfactant.

Biocatalytic Synthesis of Chiral Drug Intermediates for API's (active pharmaceutical ingredients) synthesis. R. Patel[1,2], S. Parekh[2], [1]SLRP Associates, Bridgewater, NJ, USA, [2]Unimark Remedies, Ltd., Mumbai, Maharashtra, 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 over expressed to make biocatalyst processes economically efficient, and enzymes with modified activity can be tailor-made. This presentation will describe the preparation of number of key chiral intermediates for the synthesis active pharmaceutical ingredients.

Identification of a Novel *Arabidopsis thaliana* Phospholipase A. G. Chen[1], M.S. Greer[1], I. Lager[2], J.L. Yilmaz[3], E. Mietkiewska[1], A.S. Carlsson[2], S. Stymne[2], R.J. Weselake[1], [1]Department of Agricultural, Food and Nutritonal Science, University of Alberta, Edmonton, Alberta, Canada, [2]Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden, [3]Scandinavian Biotechnology Research, Alnarp, Sweden

Plant phospholipase As (PLAs) are a complex and important group of enzymes that catalyze the hydrolyzes of fatty acids from phospholipids and have important roles in a wide range of physiological processes. We isolated a previously uncharacterized Arabidopsis lecithin:cholesterol acyltransferase (LCAT) family cDNA, linked the coding regions to the *GAL1*-inducible promoter in the yeast expression vector pYES2.1 and functionally expressed the cDNA in wild type *Saccharomyces cerevisiae*. This cDNA was demonstrated to encode a novel PLA. Although this LCAT-like PLA (LCAT-PLA) has a preference for the *sn*-2 position of phosphatidylcholine, it is evolutionally distinct from all other PLA_2 members, but shares substantial homology with mammalian lysosomal group XV PLA_2 and the strict *Arabidopsis thaliana* (*At*) LCAT-PLA_1. This novel PLA was mainly localized in the cytosol with head group specificity for phosphatidylcholine > phosphatidic acid > phosphatidylethanolamine > phosphatidylglycerol > phosphatidylserine and acyl chain specificity for oleoyl > linoleoyl > ricinoleoyl. Activity was optimal at pH 5.0 and unaffected by Ca^{2+}. The expression of *AtLCAT-PLA* in yeast inhibited cell growth and fatty acid accumulation. *AtLCAT-PLA* transcript in Arabidopsis was detected at low levels in stems, leaves and flowers, but was present at high levels in roots and siliques.

Applications of Castor Oil and the Castor Oil Plant. T.A. McKeon, USDA-ARS, WRRC, Albany, CA USA

Production and Conversion of Functional Carotenoids by Bacteria. Masashi Hosokawa[1], Kentaro Nishida[1], Tomoo Sawabe[1], Kazuo Miyashita[1], Ching T. Hou[2], [1]Hokkaido University, 3–1–1 Minato, Hakodate, Hokkaido 041-8611, Japan, [2]USDA, ARS, NCAUR, USA

Carotenoids are red, orange and yellow color pigments composed of isoprenyl units. They are well known to have beneficial health effects including anti–oxidant activity, anti–cardiovascular disease and anti–cancer effects. These carotenoids are mainly synthesized in plants and photosynthetic algae and cyanobacteria, but not in animals. On the

other hand, nonphototrophic microorganisms are observed infrequently producing carotenoids. By screening microbes, we isolated several pigmented bacteria which produce yellow, orange and pink pigments. Among them, *Algibacter lectus* produced zeaxanthin at more than 95% of its crude extracts by HPLC analysis. Zeaxanthin is dihydroxy–form of β–carotene. Zeaxanthin and lutein are normally found in human eye and prevents age–related muscular degeneration and light–induced photoreceptor cell death. The addition of glucose in the culture media increased Zeaxanthin production by *Algibacter lectus* however; the addition of sucrose or maltose decreased the production. We also succeeded in increasing lycopene production by mutation of *Algibacter lectus* treated with ethyl methanesulfonate (EMS).

Optimization of Environmental Conditions for Production of a Novel Cold-active Lipase from Pichia lynferdii Y-7723. S.Y. Park[1], M.H. Kwon[1], C.T. Hou[2], H.R. Kim[1], [1]School of Food Science and Biotechnology, Daegu, Korea, [2]Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL, USA

Lipases with abnormal functionality such as high thermostability and optimal activity at extreme conditions gain special attentions because of their applicability in the restricted reaction conditions. In particular, cold-active lipase(CAL)s have gained special attentions in various industrial fields such as washer detergent, pharmaceutical catalyst, and production of structured lipid. However, source of CAL is mostly limited in psychrophilic microorganisms. Previously we have reported about finding a novel cold-active lipase from Pichia lynferdii Y-7723 which is mesophilic yeast strain (Production of a Novel Cold-Active Lipase from Pichia lynferdii Y–7723, Hak-Ryul Kim et al., J. Agric. Food Chem. 2010, 58, 1322-1326). In this study, we investigated about optimization of fermentation conditions for production of a novel cold-active lipase from P. lynferdii Y–7723. Based on optimization study, optimal lipase productivity was obtained at 330-360 hour incubation at 20oC with 2% oil substrate in a medium composed of fructose as a carbon source. Among carbon sources tested, fructose showed almost three times high lipase production as the control medium while cell growth was similar. Yeast extract was most effective nitrogen source.

Unusual Sterol Production by Oleaginous Fungus *Mortierella alpina*. E. Sakuradani[1], Y. Fukuoka[1], S. Shimizu[1,2], J. Ogawa[1], [1]Kyoto University, Kyoto, Japan, [2]Kyoto Gakuen University, Kameoka, Kyoto, Japan

In general, cholesterol is typical in mammals, ergosterol is common in fungi and yeasts, and sitosterol, campesterol, and stigmasterol are main sterols in plants. These end sterols are composed of a similar four-ringed structure including one double bond at the Δ5 position on ring B and a specific branched side chain. Ergosterol, unlike other end sterols, contains an additional double bond at the Δ7 position on ring B. Oleaginous fungus *Mortierella alpina* is very famous as a best producer of arachidonic acid which is a precursor of eicosanoids. *M. alpina* is also known to accumulate some 24-methyl sterols and a large amount of desmosterol. Desmosterol as an unusual fungal sterol possesses one double bond at the Δ5 position on ring B, differing from the structure of ergosterol.We isolated mutants from *M. alpina* by using a chemical mutagen, which were likely to be defective in factors involved in sterol biosynthetic pathways. We identified the structures of accumulated unusual sterols which were thought to be intermediates on desmosterol biosynthesis. Furthermore, we isolated and characterized some genes involved in sterol biosynthetic pathways in *M. alpina*. Based on these results, sterol biosynthetic pathways in *M. alpina* have been considered. We also tried to improve sterol productivity by molecular breeding.

TUESDAY

MORNING

BIO 2: Biocatalysis II

Chair(s): C. Hou, Renewable Product Technology Research Unit, NCAUR, ARS, USDA, USA; and S.H. Yoon, Korea Food Research Institute, Korea

Production of Structured Lipids Using Palm Oil and its Application. S.H. Yoon, Korea Food Research Institute, Seongnam–Si, Kyunggi-Do, Korea

Human milk fat was reported as the composition of 20–25% palmitic acid, and over 70% of the palmitic acid is esterified at sn-2 position of glycerol. Human milk fat analogue was synthesized from edible animal and plant oils using a 1,3–specific lipase (Lipozyme IM 20, Rhizomucor miehei). For this study, enzymatic interesterification was carried out in organic solvent using tripalmitin and oleic acid as a model system. Optimum conditions for interesterification reaction were as follows; Molar ratio of tripalmitin:oleic acid was 1:5. Initial water content was added as 0.01 g/L, and removed after 1 h. The reaction was equilibrium after 12 h. In interesterification of tripalmitin and oleic acid, reaction products contained about 53% of 1,3–dioleoyl–2–palmitoyl glycerol (OPO). While OPO produced about 29% in intersterification of tripalmitin and triolein. Interesterification with palm and olive oil, palm and camellia oil, was relatively high value as yielded 43 and 50% of OPO, respectively. The esterification yield for palm and camellia oil was increased by concentration of OPO using melting point differences up to 70%.

Synthesis and Characterization of Acylated Amino Acids: Potential Bioactive Oleochemicals. Idris Zainab[1], Samsudin Mohd Wahid[2], Abu Hassan Hazimah[1], [1]Malaysian Palm Oil Board, Malaysia, [2]Universiti Kebangsaan Malaysia, Malaysia

A variety of amino acids and their ethyl esters were acylated with acyl chlorides under conventional and modified Schotten–Bauman conditions. Azeloyl diglycinate ethyl ester was produced through acylation reaction of azeloyl dichloride and glycine ethyl ester hydrochloride in the presence of pyridine as the organic base and in aprotic solvents. The product was a white flaky crystal with a purity of above 95% with 50% recovery after recrystallization. This process was also applicable to L–valine ethyl ester hydrochloride, L–alanine ethyl ester hydrochloride, L–leucine ethyl ester hydrochloride and L–glutamic acid diethyl ester hydrochlorides giving 76.4% to 87.0% crude yield with purity in between 81.8 to 86.7%. With L–cysteine ethyl ester hydrochloride and L–methionine ethyl ester hydrochloride as nucleophile, the reactions gave 75 and 77% crude yield, respectively. Attempts were also made to synthesize and characterize the possible products for the acylation of L–hydroxyproline with palmitoyl chloride in 50% acetone solution at 1:2 and 1:2.5 mole ratios. The components in the crude product were isolated through preparative HPLC.

Directed Interesterification of Palm Oil. Noor Lida Habi Mat Dian[1], Miskandar Mat Sahri[1], Tan Chin Ping[2], Lai Oi Ming[2], [1]Malaysian Palm Oil Board, Kajang, Selangor, Malaysia, [2]Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

Palm oil (PO) composes of about equal parts of saturated (SAFA) and unsaturated fatty acids. Its key disadvantage in competing with other oils as a food oil is that even the liquid fraction of PO, i.e., palm olein (POo), contains too much SAFA (>45%). This paper reports on results of directed interesterification (DIE) of PO to produce value–added PO fraction(s) having high oleic acid content (HOPOo) using Lipozyme TLIM lipase as catalyst. High concentrations of oleic acid can lower blood levels of cholesterol, and raise levels of high–density lipoproteins while lowering ?bad? low–density lipoproteins. Various reaction temperatures and times were investigated to achieve directed interesterified (DIEed) PO with high triunsaturated (U3) triglycerides (TAGs) content. The DIEed PO showed a very significant increase in U3 TAGs, up to 5 fold, as compared to refined, bleached and deodorized (RBD) PO. Fractionation of the DIEed PO resulted in liquid POo fraction having high oleic acid and high IV, way higher than the oleic acid content and IV of olein fraction of RBD PO. The oleic acid content and IV value of the HOPOo varied depending on the fractionation temperatures. Low IV and high melting palm stearin and diacylglycerols were the byproducts of the fractionation of DIEed PO.

Synthesis of 1,3–dicapryloyl–2–docosahexaenoylglycerol by Lipase Reaction. Y. Yamauchi–Sato, H. Uehara, S. Negishi, The Nisshin OilliO Group, Ltd., 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.

High Oxidative Stability of Functional Lipids during Fermentation of Marine Products. Naohiro Hamaoka[1,2], Masashi Hosokawa[1], Kazuo Miyashita[1], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan, [2]Food Processing Research Center, Ebetsu, Hokkaido, Japan

We have successfully developed a novel fermentation seafood paste, called "Hokkaido Sakana–miso" from fresh fish, rice malts, salts and yeasts. The procedure is based on the Japanese Miso fermentation technology. The fermentation was carried out at 30 degrees Celsius for 60 days in a barrel. Chemical analysis showed the high content of free soluble amino acids such as glycine and glutamic acid. Sensory analysis indicated that this fermentation paste is acceptable and shows a good aroma like traditional Japanese Miso. When a fermentated food, "Hokkaido Sakana–miso", was made from scallop eggs, it contained a large amount of omega–3 PUFAs (EPA and DHA) and pectenolones, originated from scallop egg lipids. These compounds have been reported to show several kinds of beneficial health effects. Although the paste contained high level of omega–3 polyunsaturated fatty acids (PUFAs), little lipid oxidation has been found during fermentation procedure and storage, suggesting the presence of antioxidant compounds in the paste. The extracts from the paste showed DPPH radical scavenging activity. This effect would be partly due to the peptides formed during fermentation from fish proteins.

Biosynthesis and Function of Polyunsaturated Fatty Acids in Thraustochytrid. T. Aki, H. Iwasaka, H. Adachi, S. Kawamoto, K. Ono, Hiroshima University, Higashi-Hiroshima, Japan

Thraustochytrids, the heterotrophic protists belonging to the kingdom Chromista, are promising industrial producer of functional lipids such as polyunsaturated fatty acids and terpenoids like astaxanthin and squalene. Studies for more efficient production by our isolates (genus *Aurantiochytrium*) and mutants as well as their utilization for industrial waste recycling and aquaculture have been conducted. Moreover, genetic manipulation method has been established with integration of exogenous genes into genomic DNA of *Aurantiochytrium* cells. Disruption of a gene encoding polyunsaturated fatty acid synthase, similar to polyketide synthase, resulted in obtaining transformants that showed a strict auxotrophy to unsaturated fatty acids. Interestingly, differential effect of various unsaturated fatty acids on complementation of the auxotrophy was observed. Besides, mutational analysis on a functional domain of the synthase might reveal the biosynthetic machinery of *cis*–double bond formation on fatty acid backbone.

Production and Characterization of Structured Lipids Containing Palmitic Acid and DHA or GLA by Lipase–Catalyzed Acidolysis for Possible Use as Human Milk Fat Analogs. S. Teichert, C. Akoh, University of Georgia, Athens, GA, USA

Structured lipids (SLs) were produced from stearidonic acid (SDA) soybean oil pre–enriched with palmitic acid (PA) at the sn–2 position with Novozym 435 (NSL) or Lipozyme TL IM (LSL) and then were improved by incorporating γ–linolenic acid (GLA) or docosahexaenoic acid (DHA). Small–scale reactions were performed to determine optimal conditions to retain sn–2 PA and incorporate approximately 10% DHA or GLA. The optimal conditions were 1:1 substrate mole ratio (NSL/LSL:DHA) for 24 h for the DHA SLs and 1:0.5 substrate mole ratio (NSL/LSL:GLA) for 12 h with both reactions occurring at 65°C. Scaled–up SLs were chemically and physically characterized. The SLs all contained over 54% sn–2 PA with over 8% GLA or over 10% DHA. Iodine values were higher for the DHA SLs than the GLA SLs due to greater unsaturation. The SL containing NSL:GLA was more oxidatively stable than the other SLs

due to its increased tocopherol content. Melting and crystallization profiles varied between the GLA SLs and DHA SLs with the GLA SLs fully melting at higher temperatures. TAG molecular species differed between the DHA SLs but were similar between the GLA SLs. Antioxidants addition stabilized the SLs. These SLs could be used as human milk fat analogs.

Novel Enzyme in Lactic Acid Bacteria for Fatty Acid Conversion to Hydroxy Fatty Acids. Shigenobu Kishino[1,2], Si–Bum Park[2], Kenzo Yokozeki[2], Sakayu Shimizu[1,3], Jun Ogawa[3], [1]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Laboratory of Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [3]Faculty of Bio–environmental Scienc,e Kyoto Gakuen University, Kyoto, Japan

Hydroxy fatty acids are widely used as industrial materials, such as resins, waxes, nylons, plastics, cosmetics, coatings, and so on. We screened the ability of transformation of unsaturated fatty acids to functional fatty acids within lactic acid bacteria, and found that lactic acid bacteria produced conjugated linoleic acid (CLA) from linoleic acid. Conjugated fatty acids have attracted much attention as a novel type of biologically beneficial functional lipid. In particular, the unique activities of CLA have been intensively studied, showing that CLA is expected to be a potential material for pharmaceuticals and dietary supplements. Furthermore, we realized that the first reaction of linoleic acid transformation is hydration of linoleic acid to 10–hydroxy–*cis*–12–octadecenoic acid. We constructed the transformed *E. coli* with this hydratase, and analyzed this enzyme. This newly enzyme catalyzes hydratation of free form of C18 unsaturated fatty acids with delta–9 double bonds in *cis* configuration and formed 10–hydroxy octadeca fatty acids. The configuration of hydroxy group produced by this enzyme is *S* optical isomer. The conversion rate with transformed *E. coli* and 100 mM oleic acid as the substrate was over 90%. This enzyme will help the industrial process of these products to be eco–friendly.

AFTERNOON

BIO 3.1/H&N 3: Food Form and Functionality of Lipids

Chair(s): M.-C. Michalski, INRA, France; and D. Hildebrand, University of Kentucky, USA

Understanding Lipid Structures in Foods in Relation to Lipid Digestibility. Harjinder Singh, Riddet Institute, Massey University

The importance of lipids in the human diet has led to major advances in understanding the mechanisms of lipid digestion and absorption. With these advances has come new recognition that the matrix in which lipids are presented (i.e. food structure) in the diet could influence the rate of lipid digestion and hence the bioavailability of fatty acids.Lipids in natural foods occur generally as in the form of complex structure in which triglycerides particles are coated with a stabilizing layer or multi–layer of membrane phospholipids and proteins. Breaking down the surrounding structures and releasing the lipid droplets from the cells, seed bodies or whatever locating matrix, will have a profound influence on our ability to digest the lipid. In processed foods, lipids may also be incorporated within the food matrix in the form of emulsions. Here phospholipids can be used as emulsifiers, but monoacylglycerols and proteins also often feature as emulsifiers and stabilizers. This paper will review the current knowledge on the state of lipids in different foods and how these systems are modified as they traverse through the gastrointestinal tract. Particular emphasis will be placed on colloidal aspects of lipid droplets and lipid digestion.

Role of Lipid Structure and Food Matrix on Lipid Digestion and Absorption. A.J. Wright, University of Guelph, Guelph, ON, Canada

Lipids play various biological roles and their consumption has critical implications in health and disease. They also contribute to the desirable and characteristic attributes of many foods. Indeed, there is intense interest in modifying lipid composition and formulating foods so as to retain these functional properties, while optimizing potential health benefits and minimizing possible deleterious consequences associated with lipid consumption. One particularly active area of study is the impact of digestive processing on food structure, lipid digestion and absorption. Progress is being made through the applications of tools to model the gastrointestinal environment, as well as interdisciplinary and multilevel research approaches. The impact of lipid and food matrix composition and structure on digestion, nutrition and health outcomes will be highlighted using examples from our team, including structured lipids and results from simulated gastrointestinal digestion experiments of lipid–based systems in relation to bioavailability. Ongoing investigations underscore the importance of food microstructure and ingredient interactions on lipid-related nutritional outcomes. A better understanding of the relationships involved will enable the rationale design of foods to optimize health and support health authorities and professionals in making recommendations.

Enhanced Absorption of n–3 Fatty Acids from Emulsified Compared with Encapsulated Fish Oil. S. Raatz[1,2], D. Bibus[3,2], [1]USDA, Human Nutrition Research Center, Grand Forks, ND, USA, [2]University of Minnesota, Minneapolis, MN, USA, [3]Lipid Technologies, LLC, Austin, MN, USA

The omega–3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have important nutrition and disease management properties. Presently fish oil (FO) supplementation relies on capsular triglyceride. Flavored emulsified lipid preparations may provide an improved approach to FO delivery. Oil in water emulsions are hypothesized to possess advantages in the digestion and absorption of their fatty acids thus increasing the bioavailability of the active components. A randomized, crossover designed study compared absorption kinetics of EPA and DHA in 10 subjects consuming 4g of FO of emulsified FO or the parent FO in capsular form. Blood was obtained at 0, 2, 4, 8, 24 and 48 hours. During this 48–hour period, there was enhanced absorption of total n–3 and EPA (0.67±0.16%, 0.45±0.06%; $p<0.01$; 0.34±0.05%, 0.23±0.04%; $p=0.05$; emulsified FO and capsular FO, respectively) observed for the emulsified FO treatment. Our findings indicate that a single dose of emulsified FO resulted in enhanced absorption of total n–3 and EPA as evidenced by changes in phospholipid fatty acid composition compared with the capsular FO during the 48–hour observation period. Oil in water emulsions are a highly bioavailable, highly tolerable method of delivery for long chain omega–3 fatty acids from FO.

Emulsified Fat Enhances Postprandial Lipemia and Exogenous Lipid Oxidation Compared with Spread Fat in Lean and Obese Humans. M.C. Michalski[1,3], C. Vors[1,2], G. Pineau[1,2], L. Gabert[3], M. Laville[3,2], H. Vidal[2,3], [1]INRA USC1235, Lyon University, Univ Lyon-1, INSA-Lyon, CarMeN Laboratory, Villeurbanne, France, [2]INSERM U1060, Lyon University, Univ Lyon-1, CarMeN Laboratory, Oullins, France, [3]CRNH-RA, CENS, Oullins, France

Obese people present an imbalance of fat distribution linked to postprandial lipemia kinetics and resulting exogenous lipid beta–oxidation. We showed in rodents that the latter could be modified using different emulsions or bulk oil. Our objective was to show in humans that non–emulsified fat and emulsified fat can modulate lipid absorption kinetics and ultimately lipid oxidation. Therefore, 10 lean and 10 obese volunteers digested 40 g of dairy fat either emulsified in a drink (+ bread) or not (spread on bread + drink). Plasma and chylomicrons were collected during 8h of digestion. Fat labelling with ^{13}C–triacylglycerols allowed to characterize the kinetics of exogenous lipid oxidation using $^{13}CO_2$ breath test and indirect calorimetry.Spread fat resulted in a later peak of chylomicrons compared with emulsified fat. This was enhanced in obese subjects, with a much later and flatter chylomicron peak during the first 5 hours of spread fat digestion. Moreover, breath ^{13}C appeared earlier and more sharply using emulsified fat. In obese subjects, emulsion even increased cumulated exogenous lipid oxidation. Dietary fat structure in food could thus be specifically adapted using the new concept of ?slow? and ?fast? lipids in order to control postprandial lipemia and lipid beta–oxidation in obese subjects.

Effects of Supplementation of Rodent Diets with Milk Fat Globule Membrane on Lipid Metabolism and Gut Microflora in Fisher 344 Rats. Robert Ward[1], Albert Zhou[1], Korry Hintze[1], Rafael Jimenez[2], [1]Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT, USA, [2]Dairy Science Department, California Polytechnic

University, San Luis Obispo, CA, USA

Production, Characterization, and Functional Properties of Structured Triacylglycerols. C.C. Akoh, University of Georgia, Athens, GA, USA

Enzymatic synthesis of structured triacylglycerols (TAGs) is now an accepted process for designing functional for the food industry and healthful lipids for enhanced nutrition. Lipases are used in the design or synthesis of alternative lipids or analogs of existing lipids such as human milk fat analogs (HMF) for infant formula, healthful lipids containing physiologically beneficial fatty acids such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and gamma linolenic acids (GLA) or for the production of functional lipids such as cocoa butter equivalents, trans–free fats for spreads, shortenings, and margarines. Fat analogs must possess desirable physical and chemical properties for possible food and clinical applications. These characteristics must be analyzed and confirmed before the application of structured TAGs in foods and nutrition. Examples of desirable properties include product purity, identity, oxidative stability, antioxidant content, melting behavior, crystal forms and morphology, FFA, n–6/n–3 ratios, TAG molecular species, positional distribution of FAs within the TAG, and sensory properties. Structured TAGs hold great promise in formulating many food products and the consumer quest for healthy good for you fats and oils.

Omega–3 Fatty Acids: Health Benefits and Sources for such Acids. I.A. Guschina, C Bascoul-Colombo, J.L. Harwood, Cardiff School of Biosciences, Cardiff, Wales, UK

There is increasing awareness of the health benefits of dietary omega-3 polyunsaturated fatty acids. Because of the inefficient conversion of alpha–linolenic acid into the long chain metabolites, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), most attention has been paid to human epidemiological surveys as well as animal experiments using EPA and DHA. There is robust evidence that the latter are of benefit for prevalent diseases such as arthritis, dementia and cardio–vascular complaints. In addition, there is experimental evidence for possible benefit in a host of other diseases and/or psychological complaints.As a result of the above there is an escalating need for increased supplies of EPA, DHA and other omega–3 fatty acids. Traditionally, fish oils have been used but this source is not sustainable and other sources are needed. New materials and genetically manipulated crops or algae will be discussed as well as attempts to improve extraction/processing of currently–used commodities.

Progress in Producing DHA in Oilseeds Using Algal PUFA Synthases. T. Walsh[1], J. Metz[2], [1]Dow AgroSciences LLC, Indianapolis, IN, USA, [2]DSM, Boulder, CO, USA

The omega–3 long–chain polyunsaturated fatty acid docosahexaenoic acid (DHA) has well–established benefits for heart, brain and eye health. However typical western diets are deficient in this important omega–3 fatty acid, leading to an increased need for sustainable and convenient sources of DHA–containing oils. Vegetable oils that are low in saturated fatty acids and high in monounsaturated fatty acids are also considered heart-healthy. We have combined these healthy oil attributes by engineering high–oleic Omega–9 canola to produce DHA by seed expression of PUFA synthase genes from marine algae. PUFA synthases use malonyl–CoA as the sole substrate for direct de novo synthesis of DHA, rather than requiring elaboration of native fatty acids such as linoleic and linolenic acids. Thus DHA production is enabled in a vegetable oil background with low saturated and high monounsaturated fat. Progress toward engineering both canola and soybean oils to contain DHA using algal PUFA synthase genes will be described.

Tailored Triglyceride Oils for Food Industry Applications. Walt Rakitsky, Solazyme, Inc., South San Francisco, CA, USA

Solazyme, Inc. is a renewable oil and bioproducts company that transforms a range of low–cost plant–based sugars into high–value oils. Utilizing an industrial biotechnology platform that harnesses the oil–producing capabilities of microalgae, Solazyme's technology is capable of using industrial fermentation equipment to efficiently accelerate microalgae's natural oil production time to a few days. By feeding their microalgae plant sugars in dark fermentation tanks, the company is in effect utilizing ?indirect photosynthesis,? in contrast to open–pond approaches. Solazyme′s

technology allows them to optimize oil profiles– or ′tailor′ them– with different carbon chain lengths, saturation levels and functional groups to modify important oil characteristics, allowing them to address specific customer requirements, while offering superior performance characteristics to convention oils.Solazyme′s tailored nutritional oils offer the food industry opportunities to: reduce saturated fat content in food products, replace partially hydrogenated vegetable oils (PHVO) while maximizing overall nutritional benefits, increase the life of cooking/frying oils, increase shelf life in packaged food products, extend the supplies of cocoa and shea butters and provides more sustainable alternatives to palm derived products.

Stearidonic Acid (SDA) Effects on EPA Levels in Red Blood Cells. E. Krul[2], R. Mukherjea[2], S. Lemke[1], D. Goldstein[1], R. Wilkes[1], [1]Monsanto Company, St. Louis, MO, USA, [2]Solae, LLC, St. Louis, MO, USA

Stearidonic acid (SDA) is a product of delta–6 desaturase, the rate limiting enzyme in the conversion of alpha–linolenic acid (ALA) to eicosapentaenoic acid (EPA). Through biotechnology, soybeans can produce oil enriched with SDA omega–3 fatty acids. Previous studies demonstrated an increase in percent EPA in red blood cells in people consuming SDA enriched soybean oil or SDA ethyl esters. In one recent study, the effects of different doses and durations of treatment with encapsulated ethyl esters of SDA and EPA on levels of EPA in RBC was assessed. Repeated Measures Analysis showed that SDA and EPA both significantly increased % EPA in RBC and the omega–3 index compared to control. The relationship between time and EPA enrichment in RBCs appears to follow a first order kinetic model. In a second study, the efficiency of SDA when incorporated as a food ingredient on EPA enrichment of RBC membranes in healthy men and women was assessed. Food sources provided 7.5 g/day of oil in two baked bars and one beverage. Mean %EPA in RBC of 0.5% at end of treatment was significantly greater compared to control. SDA was found to increase RBC%EPA with 22% efficiency of EPA alone, similar to past studies. These studies confirmed that significant increases in RBC EPA can be achieved through the consumption of foods easily formulated to contain SDA enriched soybean oil.

Optimization of Nanoliposome Formulation Encapsulating Natural Dipeptide Antioxidant by Mixture Design. Behnoush Maherani, Elmira Arab–tehrany, Michel Linder, Institut national polytechnique de Lorraine, vandoeuvre lès nancy, Lorraine, France

Encapsulation of antioxidants by nanoliposomes could represent an ameliorative approach to overcome the problems related to a range of chemical changes in food systems,including enzymatic and chemical modification, as well as extreme pH and temperature. Mixture Design of Experiments is a technique that used to determine the optimum combination of chemical constituents that deliver a desired response. The model mixture design was created to characterize ten liposome formulations having different percentage and types of lipid(DOPC, POPC, DPPC) in their formulation. Nanoliposomes prepared by thin film hydration method, were assessed by considering their physicochemical properties.The dipeptide carnosine (fl–alanyl–L–histidine)was chosen because of its wide range of antioxidant functionality in nutritional and pharmaceutical aspects. The model proposed an optimal liposome formulation by considering the appropriate size, maximum entrapment efficiency, considered fluidity for bioactive releasing in controlled condition with desired stability. The optimal point estimated by model is: 46% DOPC,12% POPC and 42% DPPC. This model could be an impressive approach which enables theoretical understanding of optimal liposome formulation including construction of liposomes with improved stability, favorable size, expected encapsulation efficiency and controlled interaction properties.

BIO 3: Biotechnical Advances in Oilseed Improvement

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

Using Molecular Genetic Strategies to Investigate the Triacylglycerol Biosynthetic Pathway in Flax. X. Pan, R.M.P Siloto, R.J. Weselake, Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada

Flaxseed oil has outstanding amounts of α–linolenic acid (18:3) and is well–known for its many health benefits. Triacylglycerol (TAG) is the main component of vegetable oils. Type–1 acyl–CoA:diacylglycerol acyltransferase (DGAT1) is proposed to exert control over TAG accumulation. Several other enzymes, however, have also been identified, including phospholipid:diacylglycerol acyltransferases (PDAT), type–2 DGATs (DGAT2), and a soluble DGAT(DGAT3). Sequence–homology and unbiased functional screening approaches were used to investigate the lipid biosynthetic machinery in flaxseed. The sequence–homology approach identified several enzymes potentially involved with oil synthesis and revealed dissimilar genome organization for genes encoding each type of enzyme. The gene expression profile suggests that DGAT1, two DGAT2s and three PDATs are involved in seed oil synthesis in flax. Expression in yeast recombinant systems indicated that DGAT2, DGAT3 and PDAT encode functional enzymes. Some of these might be selective for substrates containing 18:3. We are currently screening of cDNA library for TAG synthases using a high–throughput detection system. Overall, this study provides substantial insight into the TAG biosynthetic machinery in flaxseed and valuable information which can be utilized towards improving oil quality of flaxseed and other oilseeds.

Metabolic Engineering of Soybeans for Increased Oil and Protein Levels. D. Hildebrand[1], R. Li[1], T. Hatanaka[2], [1]University of Kentucky, Lexington, KY, USA, [2]Kobe University, Kobe, Japan

Soybeans have been one of the world's premier sources of oil and protein and together these components make up the majority of soybean seeds at ~ 60%. There have long been efforts at increasing the oil and protein contents of soybean seeds but selection for increased oil leads to a corresponding reduction in protein levels and conversely. Known soybean germplasm and lines derived from long term recurrent selection can be 5 – 10% higher in protein and 3 – 5% higher in oil than normal seeds but not more than ~60% protein and oil. Such traits are found to be maternally influenced and apparently largely governed by the amino acid and sucrose supply to the developing seeds from the mother plant. We have been able to generate soybean lines with 5 – 10% higher protean and oil levels and this has held up for multiple generations grown both in the greenhouse and field. Further genes encoding regulatory proteins affecting hydrocarbon flow into oil have been described and are being used to further increase hydrocarbon flow into seed oil TAG will further increase oil levels. Much remains to be determined concerning factors that govern hydrocarbon partitioning among the major seed components, carbohydrate, protein and oil.

Exploring Novel Approaches for Producing Oils in Plants: The Role of CGI–58 in Plant Lipid Metabolism. S. Park[1,2], S. Gidda[3], N. Khuu[3], P. Horn[2], C. James[2], K. Chapman[2], R. Mullen[3], J. Dyer[1], [1]USDA–ARS–ALARC, Maricopa, AZ, USA, [2]University of North Texas, Denton, TX, USA, [3]University of Guelph, Guelph, Ontario, Canada

The demand of plant oils is far greater than the amount that agriculture can typically deliver, resulting in a pressing need to develop novel approaches for producing higher amounts of oil in plant biomass. Although seeds are the typical source of plant oils, there is increasing interest in determining whether oils might also be produced in non–seed plant parts, including leaves and stems. Disruption of the CGI–58 gene in Arabidopsis results in a substantial increase in the amount of lipid droplets (LDs) and triacylglycerols in the leaves, but the function of CGI–58 is unknown. In mammals, the activity of CGI–58 is regulated at least in part by its interaction with other proteins, such as lipases and perilipin, but homologs for these proteins appear to be absent in plants. In an effort to better understand the function of CGI–58 in plants, we conducted a yeast 2–hybrid assay using Arabidopsis CGI–58 as bait. Although we did not identify any obvious LD–associated proteins or lipases, we did identify several proteins known to be involved in distinct aspects of plant lipid metabolism. Evidence will be presented showing that CGI–58 modulates the level of linolenic acid as well as lipid signaling pathways in plant cells.

Increasing the Energy Density of Plant Biomass by Allocating Photosynthate from Starch to Oil in Arabidopsis and Rutabaga. Sanjaya Sanjaya[1,2], Christoph Benning[1,2], [1]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA, [2]Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI, USA

To test carbon partitioning from starch to oil in plant biomass we engineered Arabidopsis plants to overproduce WRI1 and reduced the expression of ADP–glucose pyrophosphorylase (AGPase) involved in starch biosynthesis using an

RNAi approach. The resulting transgenic lines accumulated less carbohydrate and produced up to 1% oil per DW more in vegetative tissues. Numerous oil droplets were visible in vegetative tissues. The relative contribution of TAG compared to starch to the overall energy density increased in the AGPRNAi–WRI1 double transgenic line. In addition, the transgenic Arabidopsis lines resulted in 10% oil per DW on medium supplemented with 3% sugar. Transgenic rutabaga lines with AGPRNAI–WRI1 (double gene) constructs were generated. T1 transgenic rutabaga plants accumulated up to 5% oil per DW in soil grown leaves as measured by ESI–MS.

Commercial Introduction of Quality Traits: Priming the Market With High Oleic Soybean Oils. S. Knowlton, DuPont Company, Wilmington, DE, USA

Since their introduction, agricultural traits such as insect and herbicide resistance, have dominated biotech trait introductions. The development of quality traits for the food industry has been long in development and short in experience. In the next few years, the market will test the first explosion of significantly differentiated quality traits, particularly those based in soy with modified oil compositions such as high oleic and omega3 containing oils. These traits have enormous potential to become blockbuster products based on the lower cost position of soy as well as the differentiating functionality of the products themselves. If successful, more quality traits are likely to follow, such as crops with higher oil content or improved protein. However, if adoption is limited, it will likely be a long time before other quality traits are developed and commercialized because of the significant cost in bringing these products to market. This presentation will center on the new oils coming to food service and food manufacturing markets, how they perform in comparison to their conventional counterparts, and what applications are likely to be impacted. Come hear about these exciting new traits: their commercial timelines, their nutritional impact on the western diet, and their expected benefits to the food industry.

Stearidonic Acid Content in Modified Soybean Oil was Enhanced by Lipase–ediated Acidolysis. L. Kleiner[1], L. Vazquez[2], C. Akoh[1], [1]The University of Georgia, Athens, GA, USA, [2]Institute of Food Science Research (CIAL) (CSIC-UAM), Madrid, Spain

We synthesized a structured lipid (SL) enriched in stearidonic acid (SDA, C18:4 ω-3), from modified soybean oil (MSO) originally containing ~25% SDA. Low temperature crystallization (LTC) of MSO triacylglycerols (TAG) and free fatty acids (FFA) was performed. The TAG and FFA crystallization products (LTC–TAG and LTC–FFA, respectively) had SDA contents of 48.7% and 60.8%, respectively. Enzymatic acidolysis between MSO and LTC–FFA was studied using Novozym 435 and Lipozyme TL IM lipases as biocatalysts. We studied the effects of substrate molar ratio, incubation time, solvent, and enzyme load on SDA enrichment. Equilibrium was reached at 96 and 48h for Novozym 435 and Lipozyme TL IM–catalyzed reactions, respectively. The best conditions were then applied to the acidolysis of LTC–TAG and LTC–FFA. Utilizing Lipozyme TL IM and solvent–free conditions, SLs with SDA contents of 37.61 ± 1.00% (20.86 ± 6.48% at sn–2 position) and 53.46 ± 1.85% SDA (36.37 ± 3.14% at sn–2 position) were obtained from the acidolysis reaction between MSO and LTC–FFA, and LTC–TAG and LTC–FFA, respectively. When compared to the original SDA content of MSO, this process led to a 52% and 116% increase in SDA content, respectively.

Production of Industrial Proteins in Camelina. Eliot Herman, Donald Danforth Plant Science Center, St. Louis, MO, USA

Camelina is an emerging crop closely related to oil rapeseed and to the model plant Arabidopsis. Camelina oil has the prospects of being relatively easy to engineer leveraging Arabidopsis community resources. The US military has shown that Camelina oil can be readily adapted as jet aircraft fuel. Because Camelina can be cultivated on marginal lands otherwise not productive for food crops using Camelina as a fuel crop is one solution to the problem of biofuels potentially limiting food cultivation. Remnant Camelina protein meal is rich in seed storage proteins and has been used as animal feed. Because Camelina is being developed for biofuels it would be well matched to exploit Camelina to produce feed and industrial protein co–products that would enhance the value of Camelina as a crop and to increase the efficiency of its cultivation on marginal lands by more effectively utilizing the produced biomass. Current research in our laboratory is directed at developing Camelina as a protein production platform with the flexibility to produce industrial proteins or animal feed. This talk will outline our current progress in producing modified protein content in

Camelina and to discuss the prospects for its use as an industrial protein co–product crop.

Engineering Ricinoleic Acid Synthesis and Accumulation in Safflower Seed Oil. X.–R. Zhou[1], S. Okada[2], C. Wood[1], S. Belide[1], V. Haritos[2], S. Singh[1], S. Stymne[3], A. Green[1], [1]CSIRO Plant Industry, Canberra, ACT, Australia, [2]CSIRO Ecosystem Sciences, Canberra, ACT, Australia, [3]Swedish University of Agricultural Sciences, Alnarp, S–230 53, Sweden

Hydroxy fatty acids, in particular ricinoleic acid (Δ12–hydroxy–octadecenoic acid, RA), are important industrial feedstocks. The current source of RA is castor bean, a difficult crop due to presence of toxins and allergens. There is interest in Australia to engineer production of RA in safflower to provide a more safe, reliable, productive and expandable supply of this important industrial chemical. Previous research has shown that transgenic expression of fatty acid Δ12–hydroxylase (FAH12) genes (responsible for RA synthesis) in combination with either diacylglycerol acyltransferase 2 (DGAT2) or phospholipid:diacylglycerol acyltransferase (PDAT) genes, can result in RA contents up to 30% in seed oils. Although such oils may have some commercial application potential, it will be desirable to develop much higher levels of RA to simplify extraction and reduce cost. To this end we have undertaken biochemical examinations of developing safflower embryo microsomes to define potential metabolic bottlenecks for the synthesis and accumulation of ricinoleic acid. To alleviate such bottlenecks we are exploring the introduction of genes encoding specialised triglyceride assembly enzymes obtained from plants that naturally accumulate very high levels of RA in their seed oils.

Barriers to Biotech Crop Exports: Regulatory, Sustainability, and Liability Risks. T.P. Redick[1], [1]Global Environmental Ethics Counsel, LLC, Clayton MO, USA, [2]United Soybean Board, Chesterfield, MO, USA, [3]U.S. Soybean Export Council, Chesterfield, MO, USA

This presentation would provide an overview of the pipeline of biotech crops submitted in the US for regulatory approval and discuss the barriers to entry at home and abroad. This includes "precautionary" approaches to regulatory approval, sustainability standards that discriminate against biotech crops and create liability risks in the US for disrupting overseas markets. Courts in the US are considering cases alleging biotech companies should be liable for nuisance and negligence based on the adverse economic impact of a biotech crop lacking overseas approval, and USDA has asked an advisory committee (AC 21) about "compensation funds" for organic producers. Biotech companies will find that grain traders insist that seed companies secure approval in all major overseas market, or agree to be responsible for economic liability risks. The same grain traders may see overseas sustainability standards, like the European Union's Renewable Energy Directive, as another requirement that the supply chain must meet, requiring US producers to measure their carbon footprint. These barriers to entry create opportunities for biotech crops that are more sustainable than other crops, but also could prevent the most sustainable crops from reaching the market.

WEDNESDAY

MORNING

BIO 4/S&D 4: Biobased Surfactants

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

Use of Enzymes to Prepare Biobased Surfactants: Overview. D.G. Hayes, University of Tennessee, TN, USA

Biobased surfactants, employed as emulsifiers, wetting agents, plasticizers, and agents for lowering surface and interfacial tension, are becoming increasingly popular for use in foods, cosmetics, pharmaceuticals, and other industries. This trend is driven by the increase of cost for petroleum, the enhanced environmental sustainability provided through use of renewable resources, and the increased abundance of bio–based feedstocks resulting from development of biorefineries. Although most biobased surfactants are manufactured by chemical means, their preparation via bioprocessing is very attractive for future employment due to further enhancement of sustainability and potential savings in energy, downstream purification, and disposal costs. This presentation provides an overview of current research and development to prepare biobased surfactants via enzymatic reactions in nonaqueous media using enzymes such as lipases and glucosidases.

Vegetable Oil Based Surfactants: Physical Chemistry and Performance Properties. George Smith, Huntsman Corporation, The Woodland, Texas, USA

Modern surfactants are based on either naturally derived or synthetic feedstocks. Natural surfactants are typically based on alcohols derived from coconut or palm kernel oil whereas synthetic surfactants are based on ethylene derived from gas, oil and coal. This presentation will discuss the physical chemical properties of surfactants based on vegetable oils like soy and canola as low cost, locally grown alternatives to conventional natural and synthetic based surfactants. A series of vegetable oil based surfactants were prepared by reacting different natural oils like soy and canola with polyols derived from glycerin. The properties of vegetable oil derived surfactants have been compared to more conventional natural alcohol ethoxylates (AE). In general, vegetable oil surfactants have a lower CMC, cloud point and foam potential than AEs due to the longer alkyl chain length. Surface and interfacial tension depend on the alkyl chain distribution and the degree of polymerization on the polyol. Vegetable oil derived surfactants show good detergency in single surfactant and multi–component systems.

Rhamnolipid Production and Applications. M. Sodagari, Y. Chen, S.S. Dashtbozorg, N. Callow, L.–K. Ju, The University of Akron, Akron, OH, USA

Rhamnolipids are among the best known and studied biosurfactants produced by bacteria. The glycolipid biosurfactants have many potential industrial, environmental and medical applications. The common aerobic fermentation for rhamnolipid production is complicated by the highly foaming nature of the fermentation broth. We have proposed to develop an alternative production route via denitrifying *Pseudomonas aeruginosa*. Highly productive strains have also been selected. The denitrification route, nonetheless, has also met various challenges. We will present our recent work with a productive strain by both aerobic and denitrifying processes. Under aerobic conditions about 70 g/L of rhamnolipids can be produced at the volumetric productivity of 0.35 g/L– and specific productivity of 0.023 g/g–, with a yield of about 40% from vegetable oil. Further improvement on volumetric productivity will require more fundamental breakthroughs in foaming control. A process to collect and purify the rhamnolipids from the fermentation process has been developed. We have explored applications of rhamnolipids in modification of fungal morphology, affinity foaming for enzyme purification, reduction of bacterial attachment for biofilm formation, and inclusion in wound dressing. We will briefly describe some results of these applications.

Cyanophycin–based Lipo–dipeptides as Biosurfactants. J.A. Zerkowski, D.K.Y. Solaiman, ERRC, ARS, USDA, Wyndmoor, PA, USA

Cyanophycin (Cp) is a polypeptide that can be obtained by growing cyanobacteria on renewable feedstocks such as agricultural byproducts. The structure consists primarily of an aspartic acid backbone with pendant arginines, and this beta–Asp–Arg dipeptide can be isolated enzymatically. We propose that it can serve as a useful building block for the construction of surfactants. This presentation will describe the synthesis and surfactant properties of several structural variants of the Cp dipeptide with hydrophobic chains, derived from fatty acids, attached. One, two, or three lipophilic chains can be attached at varying sites while still retaining a charged unit to aid water solubility.

Fermentative Production and Interfacial Properties of Glycolipid Biosurfactants, Cellobiose Lipids by Cryptococcus Humicola. Tomohiro Imura, Tomotake Morita, Tokuma Fukuoka, Dai Kitamoto, Research Institute for Innovation in Sustainable Chemistry, National Institute for Advanced Science and Technology (AIST), Tsukuba,

Ibaraki, Japan

Biosurfactants (BS) produced by a variety of microorganisms show unique properties compared to petroleum–based synthetic surfactants. The numerous advantages of BS have prompted applications including the food, cosmetic, and pharmaceutical industries.Among BS, glycolipid–type BS are promising, because they are abundantly produced by yeast fermentation and show unique surface–active and self–assembling properties. Cellobiose lipids (CL) have also been promising, due to the high antimicrobial activity. Recently, Cryptococcus humicola was reported to produce a new type of CL. The new CL is an asymmetric bolaform surfactant that has two different polar heads at opposite ends of the hydrophobic core, and is thus attractive from the viewpoint of interfacial properites. However, little is known on the properties of bolaform biosurfactant, due to the low production yield.We thus focused our attention on the improvement of CL production using different strains of Cr. humicola, and on the interfacial characterization of the glycolipids. Cr. humicola JCM 1461 efficiently produced the CL, tetra–acetylated cellobiose bearing 2–hydroxy–hexadecanoic acid. The present CL exhibited not only a high surface activity but also unique supramolecular gel formation in various solvents.

Synergetic Interactions among Greener Surfactants and Their Synergistic Interactions with Enzymes. P. Somasundaran, J. Wu, S. Lu, M. Chin, NSF I/UCR Center for Particulates and Surfactants, Columbia University, New York, NY, USA

Due to the increasing demand for environmentally benign reagents, greener surfactants are recently receiving the warranted attention. The unique surface activity along with their biodegradability makes this group of surfactants leading candidates as the next generation reagents. One of the unique properties of greener surfactants is the synergistic interactions between each other as well as conventional surfactants. In this work, we selected sugar based greener surfactant alkyl glycosides and conventional surfactant sodium dodecyl sulfonate to explore their potential synergism in mixtures. A range of techniques including surface tensiometry, fluorescence spectroscopy, ultrafiltration, and analytical ultracentrifugation (AUC), were employed to obtain information on the micellization behavior of the mixture. The interaction parameter, monomer concentration, micellar size and shape distribution were obtained for the mixed surfactant system as a function of total surfactant concentration as well as mixing ratio to obtain a full understanding of their aggregation behaviors. Interestingly, coexistence of two types of micelles was identified for the first time and a model is proposed to explain such coexistence based on the interactions between the two types of surfactants. Since enzymes are used along with surfactants, Interactions between a surfactants and enzymes also have been studied. Interestingly, even enhancement of surface activity was observed which was attributed to be the result of micelle/protein interactions that induce a more flexible structure that is more conducive to lowering reaction–energy barriers. These findings are useful for optimizing the composition of mixed surfactant systems and enhancing the synergetic efficiency of the system to achieve more effective and economical formulations.

Sophorolipids and Sophorolactone: Properties and Application Potential. D.W.G. Develter, Ecover Belgium NV, Belgium

Glycolipids can be produced by bioconversion of native and renewable feedstocks such as rapeseed oil. Sophorolipids for example are currently finding their way to the detergent market. These attractive surfactants combine green oleochemistry with an impeccable environmental profile and excellent hard surface cleaning. A new to literature sophorolipid species is decribed. The interesting physicochemical behaviour of sophorolactone, their surface modification properties and some accompanying promising applications are reported.

Formulating with Bio and Biobased Surfactants. E. Acosta, M. Baxter, University of Toronto, Toronto, ON, Canada

Formulating or reformulating products to incorporate bio or bio–based surfactants is, in most cases, not a trivial task. In this presentation we will explain the use of the HLD–NAC framework to characterize the hydrophobicity of bio and bio-based surfactants and the design of surfactant mixtures to achieve specific performance targets. Literature examples of formulations used in pharmaceutical and environmental applications will be used to illustrate these principles. Finally, an example on the characterization and use of surfactants extracted from waste biomass will be discussed.

Home Care Cleaning Products based on Renewable Materials with Novel Added Benefits. P. Stuut, Purac, Purac, Gorinchem, Zuid–Holland, the Netherlands

Within the home care area there is a clear and continuous trend towards more safe and sustainable ingredients and detergent formulations. Consumers expect safer products and more environmentally friendly products. The origin of more and more raw materials for home care and industrial products can be found in renewable feedstocks like sugars and starches. From these feedstocks, biobased products like lactic acid and derivatives can be produced. They bring added benefits and have a positive impact on CO^2 emission reduction. Next to this, these products are generally biodegradable and safe for humans and the environment.Lactic acid is currently used in a wide variety of home care products because of its functional benefits in cleaning, descaling and anti–bacterial properties. Current products include toilet bowl cleaners and dish wash products. Recently a novel product for the home care and industrial cleaning market has been developed. This novel product is solid, is mixable with other ingredients like surfactants and perfumes and can be molded into different shapes. During usage the material gradually hydrolyses, delivering lactic acid and other cleaning actives to the substrate. This way, longer lasting cleaning benefits are obtained by a renewable, functional material.

Modified Activated Sludge Oil Phospholipids as Potential Bio–based Surfactants. P.J. Pham, R. Hernandez, W.T. French, W. Holmes, Dave C. Swalm School of Chemical Engineering Mississippi State University, Mississippi State, MS USA

Phospholipids extracted from activated sludge oil comprise about 20–25%. ^{31} P–NMR revealed that the phospholipid profile consists of a variety of phospholipids with phosphatidylethanolamine (PE) present in majority (17–18%) followed by phosphatidylglycerol (4%) and phosphatidylcholine (5%). Chemical modification of the phosphatidylethanolamine head group was primarily accomplished by the acylation/acetylation reaction that involved acetic anhydride and an organic base, triethylamine to convert it into an acetylated form (NAc–PE). The potential as bio–based surfactants of these low polarity phospholipids were explored.

Achieving Effective, VOC Compliant, All–Purpose Cleaner Formulations Using Biorenewable Surfactant Blends. Molly I. Busby, Kirsten K. McNally, Dow Chemical Corporation, Midland, MI 48674, USA

The cleaning industry continues a drive to minimize carbon foot print by using raw materials from biorenewable sources; however, biorenewable surfactants often don't provide optimum cleaning performance by themselves. We have investigated blends of alkylpolyglucosides with DfE compliant non–ionic surfactants as a way to increase use of biorenewable surfactants in cleaning formulations. The resulting surfactant blends displayed multifunctional properties, excellent alkaline stability, solubility, degreasing, fragrance coupling and ease of formulation. These surfactant blends are suitable for concentrates to minimize shipping costs, conserve energy, provide shelf stability and minimize pollution. Examples of the use of these surfactant blends with solvents, alkaline agents, and chelants to prepare VOC compliant formulations for all purpose, hard surface, and carpet cleaners to meet the 2012 target will be discussed.

New Cyclic C–Glycoside Surfactants Derived from Carbohydrates. Neil A. Burns, P2 Science, Inc., New Haven, CT, USA

P2 is commercializing a new, patent pending, class of cyclic *C*–glycoside surfactants, made via a highly efficient, one-pot process. The chemistry adheres closely to the principles of green chemistry, including atom economy, step economy, and the use of renewable feedstocks, carbohydrates and renewable vegetable oils. Carbohydrate–based surfactants are an important class of nonionic surfactants that include alkyl polyglucosides (APGs), sorbitan esters (tradenames SPAN and Tween), and methyl ester glucosides (MEGs). In addition to being renewable, carbohydrate–based surfactants have a number of favorable attributes including desirable detergency properties and low toxicity. However, current carbohydrate–based surfactants are generally unstable in due to the use of either a base–labile ester linkage (SPAN, Tween, and MEGs), or an acid–labile O–glycoside linkage (APGs) in their structures. P2's *C*–glycosides replace these traditional bonds with a more stable *Carbon – Carbon* bond. The resulting family of surfactants, is extremely stable across a wide range of pH and temperature conditions, while performing as extremely

efficient surface tension reducers over a full range of HLB's. Applications include household and I&I cleaning, personal care, cosmetics and a range of industrial applications including oilfield, emulsion polymerization, lubricants and agrochemicals.

AFTERNOON

BIO 5: General Biotechnology

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

Synthesis of Structured Lipid Enriched in LCPUFA from Palm Olein for Infant Formula and Nutraceutical Use. S. Nagachinta, C.C. Akoh, Department of Food Science and Technology, UGA, Athens, GA, USA

Long–chain polyunsaturated fatty acids (LCPUFA) such as docosahexaenoic (DHA) and arachidonic (ARA) acids have great benefits for the development and maintenance of human brain function and cognition. This study aims to modify the structure of palm olein, a common oil used in infant formula to incorporate DHA and ARA as well as increase the amount of palmitic acid at the *sn*–2 position. Enzymatic acidolysis using Novozym 435® at different conditions (time, temperature, and substrate mole ratio) were optimized using response surface methodology. Concentrated DHA and ARA were obtained through saponification and urea complexation of single cell oils. The optimal condition resulted in 25.25% total incorporation of DHA+ARA and 17.20% DHA+ARA incorporation at the *sn*–2 position. The amount of palmitic acid at the *sn*–2 was 22.11%. The content of palmitic, oleic, and linoleic acids in the structured lipid were comparable to the values of human milk fat (23.9, 35.3, and 9.0%, respectively). The structured lipid produced in this study has potential for use in infant formulas as well as in nutraceutical applications for pregnant women.

Lipid Production from Yarrowia lipolytica Po1g Grown in Sugarcane Bagasse Hydrolysate. Yeshitila Asteraye Tsigie[1], Chun-Yuan Wang[1], Chi-Thanh Truong[2], Yi-Hsu Ju[1], [1]National Taiwan University of Science and Technology,Department of Chemical Engineering, Taipei, Taiwan, [2]Cantho University, Department of Chemical Engineering, Cantho City, Vietnam

This study investigated the possibility of utilizing detoxified sugarcane bagasse hydrolysate (DSCBH) as an alternative carbon source to culture Yarrowia lipolytica Po1g for microbial oil and biodiesel production. Sugarcane bagasse hydrolysis with 2.5% HCl resulted in maximum total sugar concentration (21.38 g/L) in which 13.59 g/L is xylose, 3.98 g/L is glucose, and 2.78 g/L is arabinose. Detoxification of SCBH by Ca(OH)2 neutralization reduced the concentration of 5–hydroxymethylfurfural and furfural by 21.31% and 24.84%, respectively. Growth of Yarrowia lipolytica Po1g in DSCBH with peptone as the nitrogen source gave maximum biomass concentration (11.42 g/L) compared to NH4NO3 (6.49 g/L). With peptone as the nitrogen source, DSCBH resulted in better biomass concentration than Dglucose (10.19 g/L), D–Xylose (9.89 g/L) and NDSCBH (5.88 g/L). The maximum lipid content, lipid yield and lipid productivity of Yarrowia lipolytica Po1g grown in DSCBH and peptone was 58.5%, 6.68 g/L and 1.76 g/L–day, respectively.

Batch and Continuous Production of Microbial Oils as Biodiesel Feedstock from Hydrolyzate of Switchgrass. Guochang Zhang, William T. French, Rafael Hernandez, William E. Holmes, Dave C. Swalm School of Chemical Engineering, 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. Microbial oils derived from lignocellulosic biomass could potentially be a cheap source of biodiesel. The objective of this investigation is to optimize microbial oil production by

Rhodotorula glutinis (ATCC 15125) using hydrolyzate of switchgrss as sole carbon and energy source. In the experiments, the C to N ratio and C to P ratio as well as the temperature were evaluated on the biomass growth and lipid production of R. glutinis. The kinetic models were also studied for the biomass growth and lipid accumulation. In the experiments, both cell growth and lipid accumulation were investigated using the media made of hydrolyzate of switchgrass. Sugar concentration in culture was determined by HPLC–ELSD method. The lipid content in the cells was measured by gravimetrical Bligh & Dyer extraction. The biomass concentration reached 5.37 g/L with lipid content in the cell increasing from initial 1.6% (wt%) to 21.35% within 196 hours at 3°C when the C/N ration was controlled above 55:1. The majority contents of triacylglycerides were characterized by high temperature gas chromatography to be of C16~C18.

Generation of Renewable Fuels and Carotenoids from *Rhodotorula glutinis* using Sweet Sorghum Juice. M. Revellame[1], D. Sparks[1], R. Hernandez[2], W. Holmes[2], A. Brown[1], [1]Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Mississippi State, MS,USA, [2]Dave C. Swalm School of Chemical Engineering, Mississippi State University, Mississippi State, MS,USA

Rhodotorula glutinis is an oleaginous, oxidative yeast that has the ability to produce up to 70 % of its weight as oil in the form of triacylglycerol (TAG). Additionally, it accumulates carotenoids, which act as antioxidants to protect molecular structures and provide pigmentation to cells. This microorganism is cultivated using sweet sorghum juice containing 17.0 % total sugars composed of glucose, fructose and sucrose for seven days. The lipids were extracted using a modified Bligh and Dyer lipid extraction method followed by transesterification. The resulting fatty acid methyl esters were analyzed by gas chromatography equipped with flame ionization detection. Carotenoids were extracted with petroleum ether and quantified using high performance liquid chromatography with diode array detection. The cultivations were done at pH 2.5, 5.5 and 7.5 and carbon to nitrogen of 10, 40 and 70. The results showed that at pH 5.5 and a carbon to nitrogen ratio of 70, the growth is favorable for the yeast and resulted in the highest amount of total fatty acid methyl esters consisting primarily of oleic, linoleic, and palmitic acid methyl esters. The carotenoids on the other hand, particularly β-carotene was at maximum at pH 5.5 and but C: N ratio of 10.

Conversion of Glycerol to 1,3–Propanediol Using Ethanol Stillage. Kornsulee Ratanapariyanuch[1], Youn Young Shim[2], Monique Haakensen[3], Martin Reaney[2], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [3]Contango Strategies Limited, Saskatoon, Saskatchewan, Canada

Billions of liters of stillage, a byproduct of the ethanol industry, are produced annually in ethanol plants in the USA and Brazil. Stillage is a dilute aqueous mixture of organic and inorganic molecules. Typical organic constituents include glycerol, 1,3–propanediol, glycerolphosphorylcholine (GPC), betaine, lactic acid, acetic acid, and phenethyl alcohol. These compounds are potentially valuable without modification or as precursors for additional processing. It was observed that storing stillage solution at 32°C led to conversion of the glycerol and lactic acid in the stillage to 1,3–propanediol and acetic acid, respectively. Interestingly, when strains of Lactobacillus bacteria were isolated from the fermenting stillage solution, cultured and reintroduced to the stillage, improved conversion of glycerol to 1,3–propanediol was observed. Here we report the modification of glycerol present in stillage by bacterial fermentation with strains of L. panis. In addition we describe the conversion of glycerol, from a biodiesel source, added to the stillage. The conversion of glycerol from both biodiesel and ethanol production to 1,3–propanediol may be achieved.

Development of a Novel Bioprocess to Produce Adipic Acid from Renewable Oils. T.A. Beardslee, M. Walbridge, J. Yi, S. Picataggio, Verdezyne, Inc., Carlsbad, CA, USA

Adipic acid is an important industrial chemical used to make Nylon 6,6 and polyurethane resins. It is currently produced from petrochemical sources with an estimated global market of 5 billion pounds per year. The production of adipic acid from renewable resources would allow the production of completely bio–based nylon and polyurethanes. To this end, we have engineered the diploid yeast Candida tropicalis to produce adipic acid from plant–based oils. C. tropicalis can normally grow on alkanes or fatty acids as the sole carbon source via cyclic degradation through its beta–oxidation pathway. A strain in which this pathway has been completely blocked can convert these substrates at high

yield and selectivity to the corresponding dicarboxylic acids via the omega–oxidation pathway, producing diacids with a chain–length distribution that precisely mimics the plant–based oil feedstock. We have engineered both the beta–oxidation and omega–oxidation pathways to enable selective production of adipic acid from any plant–based oil, regardless of its fatty acid composition.

Protein Engineering of Lipases to Alter Their Fatty Acid Selectivity. U.T. Bornscheuer, H.B. Brundiek, A.S. Evitt, R. Kourist, Institute of Biochemistry, Greifswald, Germany

Protein engineering has developed in the past decade to an important technology to alter the properties of enzymes [1,2]. Whereas initially rational protein design was the method of choice, directed evolution (in essence a random mutagenesis followed by screening or selection of desired mutants) became an important alternative. More recently, researchers use combinations of both methods. In this lecture, the principle strategies and current challenges in protein engineering will be highlighted. We applied protein engineering to alter the fatty acid selectivity of lipase A from Candida antarctica [3]. The strategy used and results obtained will be presented.[1] Kazlauskas, R.J., Bornscheuer, U.T. (2009) Nature Chem. Biol., 5, 526-529[2] Lutz, S., Bornscheuer, U.T. (Eds.) (2009) Protein Engineering Handbook, Wiley-VCH, Weinheim[3] Brundiek, H.B., Evitt, A.S., Kourist, R., Bornscheuer, U.T. (2011), Creation of a highly trans fatty acid selective lipase by protein engineering, Angew. Chem. Int. Ed., accepted.

Specific Production of Polyunsaturated Fatty Acid by Oleaginous Filamentous Fungus *Mortierella alpina* Breeding. Akinori Ando[1], Tomoyo Okuda[1], Hiroshi Kikukawa[1], Eiji Sakuradani[1], Jun Shima[1], Jun Ogawa[1], Sakayu Shimizu[1,2], [1]Kyoto University, Kyoto, Japan, [2]Kyoto Gakuen University, Kyoto, Japan

A filamentous fungus, *Mortierella alpina* 1S–4, belonging to the Zygomycetes, has been isolated from soil as a potent producer of polyunsaturated fatty acids (PUFAs) in our laboratory and used for commercial production of arachidonic acid (AA, 20:4n–6). A host-system for *M. alpina* 1S–4 was developed by means of molecular breeding for improving and modifying PUFAs productivity and composition. 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, 20:5–3) production, which was an end product of ω3 fatty acids synthesized in *M. alpina* 1–4, by overexpression of some heterologous desaturase genes.

Biotechnology Posters

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

Preparation of Cocoa Butter Equivalents by Blending a Fractionated Palm Stearin with Shea Stearin. Hyeonjin Jeon[1], Kyoung Kyu Kang[1], In-Hwan Kim[2], Hee-Don Choi[3], Chan Lee[1], Byung Hee Kim[1], [1]Chung-Ang University, Anseong, Gyeonggi-Do, Republic of Korea, [2]Korea University, Chungneung-Dong, Sungbuk-Gu, Seoul, Republic of Korea, [3]Korea Food Research Institute, Seongnam, Gyeonggi-Do, Republic of Korea

Cocoa butter equivalents (CBE) are edible fats which can replace natural cocoa butter fully in the manufacture of chocolate products. The aim of this study was to assess the possibility for the commercial use of CBE produced in our previous study. CBE were prepared by blending a fractionated palm stearin and shea stearin in a weight ratio of 40:60. CBE were blended with cocoa butter in weight ratios of 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10. The blends were evaluated for their triacylglycerol compositions, DSC thermal melting and crystallization behaviors, and solid fat contents. All blends contain 83.0–86.7% total symmetric monounsaturated triacylglycerols. They completed the melting and started the crystallization at similar temperatures to cocoa butter, respectively. However, of the 10 kinds of blends, only the 5:95, 10:90, 20:80 and 30:70 blends showed similar changes in solid fat contents as a function of temperature to cocoa butter. These results indicate that the CBE can be blended with cocoa butter to the extent of 30% without significantly altering its physical properties.

Conversion of Rice Straw to Sugars by Microbial Hydrolysis using Bacteria Isolated from Thai Higher Termites.

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Lignocellulosic biomass such as rice straw is one of the most abundant biomass in the world. Cellulose, hemicellulose and lignin of rice straw are about 47%, 25% and 5%, respectively. The cellulose and hemicellulose of rice straw can be hydrolyzed via enzymatic hydrolysis into glucose and other fermentable sugars. These sugars can be served as feedstocks for bioethanol production. The purpose of this work was to investigate the possibility of using rice straw as a raw material for enzymatic hydrolysis to produce sugars using bacteria isolated from Thai higher termites, Microceroterms sp. The effects of particle size (40 mesh, 60 mesh and 80 mesh), hydrolysis temperature (30°C and 37°C) and bacteria strain (A002 and M015) were investigated in order to determine the optimum conditions for a maximum sugar production. The results showed that the maximum sugar production was obtained with strain A002 bacteria and 90 mesh size of rice straw at 37°C.

Enrichment of Erucic Acid from Crambe Oil in a Recirculated Packed Bed Reactor via Lipase–Catalyzed Ethanolysis.

Da Som No, TingTing Zhao, Seung In Hong, Sung Won Yoon, In-Hwan Kim, Department of Food & Nutrition, Korea University, Seoul, Republic of Korea

Erucic acid enrichment was produced successfully from crambe oil in a recirculated packed bed (RPBR) reactor via ethanolysis using Novozym 435 lipase from *Candida antarctica* as a biocatalyst. The content of erucic acid of the crambe oil used was 56 mol%. The erucic acid was located predominantly in the *sn*–1,3 position of the triacylglycerol of crambe oil. Effect of reaction temperature, molar ratio, and residence time of substrate in RPBR on the erucic acid enrichment as a function of reaction time were studied. Optimal temperature, molar ratio (crambe oil to ethanol), and residence time of substrate in RPBR were 45^{o}C, 1:80, and 4 min, respectively. The maximum content of erucic acid of ca.83 mol% was obtained at the optimal condition after 30 min reaction time.

Enzymatic Process for Preparing Linoleic Acid from Passiflora alata Oil Used as Skin Whitening Agent.

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Linoleic acid is the primary component of Passiflora alata oil, a botanical compound coming from Brazilian biodiversity. The first step for preparing linoleic acid is the enzymatic splitting of Passiflora alata oil for obtaining fatty acids and glycerol by using lipases. The enzymatic splitting of oil is achieved in three steps with the addition of enzyme and water. The second step is a molecular distillation, to increase the free fatty acid content. Linoleic acid, as well as an extract of Schinus terebinthifolius Raddi, another botanical compound coming from Brazilian biodiversity, was evaluated for skin whitening qualities. The whitening effect of these compounds was assessed using specific biochemical assays designed to determine tyrosinase activity and melanogenesis in B16 cells and in in vitro models including Skin Whitening Activity on Human Reconstituted Dark–tanned Epidermis. The results showed that these natural compounds are able to reduce the tyrosinase activity in vitro as well as to decrease the level of melanin produced by B16 cells cultured with melanocyte–stimulating hormone, and reduce melanin in tanned human reconstituted epidermis models treated with the compounds. The combination of the compounds provided a synergic positive whitening effect, comparable to classical molecules used for skin whitening.

Sugars Evolution from Cassava Residue by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites.

Pitcha Wongkeo[1], Pramoch Rangsunvijit[1,2], Sumeth Chavadej[1,2], [1]The Petroleum and Petrochemical College, Chulalongkorn University, Bangkok, Thailand, [2]Center of Excellence for Petroleum, Petrochemicals, and Advanced

Materials, Chulalongkorn University Bangkok, Thailand, Bangkok, Thailand

The possibility of using cassava residue from bioethanol plant containing 47.04% starch, 23.00% cellulose, 13.64% hemicellulose, and 22.58% lignin as a raw material to produce sugars using enzymatic hydrolysis was investigated. In the experiments, each reactor contained cassava residue, bacteria cells, and production medium. The effects of particles size, temperature, and strains of bacteria isolated from Thai higher termites, Microcerotermes sp., which are 40 mesh, 60 mesh, and 80 mesh, 30°C and 37°C, and strain A 002 and M 015, respectively on the glucose concentration were focused. In addition, effects of the malt extract quantity in 65 modified DSMZ broth medium 2 on the sugars concentration was determined. High performance liquid chromatography (HPLC) with a refractive index detector was used to determine the quantity of sugars. The maximum sugars production was obtained at 37°C using strain A002 and 80 mesh of cassava residue.

Isolation of Giant Panda Intestinal Microbes to be used in an Oil-based Biofuel Platform.

C. Williams[1], C. Johnston[1], A. Kouba[2], S. Willard[1], D. Sparks[1], A. Brown[1], [1]Mississippi State University, USA, [2]Memphis Zoological Society, USA

The use of giant panda feces may hold the key to reducing the cost of biofuel production. The giant panda's fecal metagenome has elucidated multiple cellulolytic microorganisms that can be used in the conversion of lignocellulosic biomass–based biofuels, and preliminary evidence suggests that oleaginous microorganisms may also be present. Twelve species of cellulolytic microbes, including *Clostridium cellulovorans*, *Clostridium phytofermentans* and *Pseudomonas fluorescens* were isolated using Ilumina sequencing. These microbes can be used to pretreat lignocellulosic biomass, a waste product of other agricultural processes, converting the biomass into simple sugars that can be used by oleaginous microbes to accumulate lipids. These lipids can then be converted into renewable diesel and biodiesel; thus creating a usable product from two waste materials and lowering costs associated with biofuel production. Further characterization of the microbes is currently underway to evaluate the activity of the enzyme systems and processes associated with these metabolic pathways.

Canceled - Production of Omega-3 LC-PUFAs in Oilseed by Seed Expression of Multisubunit Microalgal PUFA Synthases.

T. Walsh, Dow AgroSciences LLC, Indianapolis, IN, USA

Enzymatic Interesterification of a Blend of Palm Stearin: Cottonseed Oil for Low *trans*–margarine Formulation.

Yingyao Wang[1,2], Lingzhi Cheong[2], Cuiping Wei[1], Zhangqun Duan[1], Xia Luan[1], [1]Academy of State Administration, China, [2]Aarhus University, Denmark

Interesterification has received increasing interest lately as an alternative to partial hydrogenation for the production of "?low trans" hard fat. An interesterified structured lipid was produced with a lipid mixture of cottonseed oil (CO) and palm stearin (PS) at five weight ratios (CO: PS from 30:70 to 70:30) using lipase (Lipozyme TL IM, 5%) as a catalyst at 65 for 24h. Transesterification did not much alter the fatty acid (FA) composition of the mixture, but the physical properties of the products changed and showed lower melting points and solid fat contents, TAG composition, different melting and crystallization behaviors as well as the formation of more stable crystals. The physical properties of the interesterifed fats were influenced by the amount of PS, resulting in more hardness and higher solid fat contents for 30:70 than the other blends. The present study suggested that the produced interesterified fats containing trans–free fatty acids could be used as alternatives to hydrogenated types of bakery margarine.

Cloning, Sequencing and Characterization of Lipase from a Polyhydroxyalkanoate– (PHA–) Synthesizing Pseudomonas resinovorans.

JeungHee Lee[3,1], KiTeak Lee[2], Daniel Solaiman[1], [1]USDA, ERRC, USA, [2]Chungnam National University, Daejeon, Korea, [3]Department of Food Nutrition, Daegu University, Jillyang Gyeongsan Gyeongbuk, South Korea

Lipase gene (lip) of a biodegradable polyhydroxyalkanoate– (PHA–) synthesizing bacterium P. resinovorans NRRL B–2649 was cloned, sequenced and characterized by PCR and genome walking method. The ORF of the putative Lip (314 a.a.) and its active site (Ser111, Asp258 and His280) forming a catalytic triad were identified. The biological function of the cloned lip gene in P. resinovorans was verified via the construction of a lip–knockout mutant by a transposon–insertion inactivation method. Unlike the wild–type, the lip–knockout mutant could not utilize TAGs (tributyrin, tallow and olive oil) due to no production of active extracellular lipase as determined by detection of fluorescence emission of cell culture and cell–free culture medium on a rhodamine B plate assay, and by TLC analysis of the composition of acylglycerols and free fatty acid in the extracts of the spent culture medium, thus establishing that the cloned lip gene is responsible for the lipase activity of P. resinovorans. Our study also showed that tributryin is more effective than olive oil for inducing lipase production in P. resinovorans. The outcome of this study contributes to the basic knowledge of the fatty acid metabolism of PHA–producing P. resinovorans that could use agricultural fats and oils to make bioplastics.

Rational Synthesis of 1,3-diolein by Enzymatic-Esterification.

Zhang–Qun Duan[1,2], Wei Du[2], De-Hua Liu[2], [1]Academy of State Administration of Grain, Beijing 100036, P.R. China, [2]Department of Chemical Engineering, Tsinghua University, Beijing 100084, P.R. China

1,3–Diacylglycerol oil has beneficial effects on suppressing the accumulation of body fat and preventing the increase of body weight. More and more attention has been paid to enzymatic 1,3–diacylglycerol production in recent years due to its mild reaction condition and safe products. *t*–Butanol was an excellent reaction medium for enzyme-mediated esterification of oleic acid with glycerol for 1,3–diolein preparation which has been proved in our previous work. Herein, to further improve the diolein yield and the value of 1,3–diolien/diolein, response surface methodology was applied to determine the effects of the significant variables and their reciprocal effects on the product synthesis. Under the optimal conditions (62.4^{o}C, 0.75 g Novozym 435, substrate molar ratio (oleic acid/glycerol) 2.4 and 4.8 g *t*-butanol), the diolein yield of 87.4% could be achieved, and the value of 1,3–diolien/diolein was as high as 87.8%. The further verification experiments confirmed the validity of the predicted model. Moreover, the enzyme still retained a remarkably high proportion (92.5%) of its original catalytic activity after 100 cycles of successive re-use under the optimum conditions. In particular, this work is focused on manipulating and controlling the process variables towards reaching the rational production of 1,3–diolein.

Production of Human Milk Fat Analogues Containing Docosahexaenoic and Arachidonic Acids by Enzymatic Reactions.

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Human milk fat analogues (HMFA) containing docosahexaenoic (DHA) and arachidonic acids (ARA) were produced by two step enzyme–catalyzed transesterification reactions. In the first part of the study, hazelnut oil was enriched with ethyl palmitate to increase palmitic acid (PA) amount at the sn–2 position by using Novozym® 435 lipase at a constant temperature of 65°C. The incorporation of PA into hazelnut oil was optimized by Response Surface Methodology at substrate molar ratio of 1:4 – 1:6 and time of 6–18 h.In the second part of the study, customized hazelnut oil with increased PA content at the sn–2 position was enriched with DHA and ARA using DHASCO® and ARASCO® single cell oils in the presence of a sn–1,3 specific lipase, Lipozyme® RM IM. After evaluation of reaction conditions, gram–scale production was performed at 60°C with substrate molar ratio of 1:0.1 and ARA/DHA ratio of 3:2 for 3 h. Structured lipid (SL) obtained at these conditions contained 57.3% PA, 2.7% ARA and 2.4% DHA. PA amount of SL at the sn–2 position was 66%.These HMFA may be incorporated into products to enhance the development and growth of infants as well as providing HMF characteristics to the vegetable oil.

Methylester Fractionation–Distillation.

John Monfre[1], Fredrik Pomrehn[2], [1]Lurgi, USA, [2]Lurgi GmbH, Frankfurt, Germany

Methylesters are either produced by "Esterification" or "Transesterification".Whilst for some raw materials a washing

process is sufficient whenever the final product is to be used as biodiesel, other raw materials require more elaborate steps like straight distillation, or in order to generate more distinct product characteristics: Distillative fractionation. Typically distillation and fractionation are undertaken under low vacuum and at temperatures of 200–250°C. A straight Methylester distillate represents the identical carbon chain distribution as the feedstock and is an almost colorless liquid. The high boiling components are retained as residue. The residue contains amongst others unconverted mono–/di–/triglycerides, physical impurities and sterol glycosides. If, for instance, the CFPP of a palm based Methylester is to be improved, a C16–fraction can be removed in a fractionation column by separation over structured packings. Typically a C16–fraction of >95% purity can be generated whilst the distillate is a composite of residual C16– and C18–fractions with a CFPP close to 0°C. Raw materials based on palmkernel oil offer a wider variety of fractional composites like C8/C10, C12/C14, C16/C18 or individual high purity fractions of around 99% purity, all with special high value applications.

Discovery and Functional Characterization of Microsomal Oleate Desaturases of Santalaceae.

S. Okada[1], X.-R. Zhou[2], N. Gibb[1], K. Damcevski[1], V. Haritos[1], [1]CSIRO Ecosystem Sciences, Acton, ACT, Australia, [2]CSIRO Plant Industry, Acton, ACT, Australia

Plants in the Santalaceae family produce ximenynic acid (trans–11–octadecen–9–ynoic acid) in the developing seed, and related polyacetylenic fatty acids in vegetative tissues of the plants. These are possibly formed by integral membrane oxidoreductases that catalyze desaturation of fatty acids.We identified 12 desaturase genes from quandong and native cherry related to the microsomal oleate desaturase represented by Arabidopsis thaliana FAD2. Phylogenic analysis showed one group of desaturases clustering with other reported FAD2s, whereas the other two groups formed their own unique cluster separated from both FAD2 and other divergent relatives. Functional characterization found the quandong FAD2 and native cherry FAD2–1 to have Î?12 desaturase activity, but also Î?11 conjugase activity on stearolic acid (octadecen–9–ynoic acid), thus producing ximenynic acid. Of the two divergent FAD2 clusters, FADX from both Santalaceae species added a terminal double bond to stearolic acid, showing their preference to recognize an acetylenic fatty acid substrate. The pathway of ximenynic acid production in the Santalaceae is discussed in light of these results.

Evaluation of Deep Eutectic Solvents as New Media for iCALB–catalyzed Reactions.

Erwann Durand, Jérôme Lecomte, Bruno Barea, Georges Piombo, Pierre Villeneuve, UMR IATE CIRAD, Montpellier, France

Some lipases preserve good activity in organic solvent and their ability to catalyze transesterification reactions in organic media is known and well documented. However, most organic solvents can be environmentally damaging or toxic. Therefore, in a context of green chemistry a number of studies have attempted to resolve this dramatic environmental impact and try to realize lipase–catalyze reaction in green solvent. Ionic liquids were the first potentially alternatives to organic solvents for biotransformation; they are non volatile, thermally stable and have high solvation properties. Nevertheless, the limitations of ionic liquids are their cost, toxicity and the need for high purity. Deep eutectic solvents (DESs) are a new class of solvent typically formed by mixing an ammonium salt and a hydrogen–ond donor. Like ionic liquids, DESs often have melting points close to room temperature, low volatility and high thermal stability. Unlike them, they are biodegradable, cheap and very easy to prepare. In this context, it will be very interesting to develop lipase–compatible DES from inexpensive and biodegradable cholinium salt. Therefore, in order to know the potential of DESs to be friendly–media for lipase–catalyzed reaction we investigated iCALB compatibility in eutectic solvents with a focus of the activity, selectivity and stability after long incubation–time.

Optimization of Conditions for Drying Oil Production in Transgenic Plants: Analysis of Relative Effects of Codon Usage, Epitope Tagging, and Promoter Choice.

J. Shockey[1], E. Cahoon[2], J. Dyer[3], [1]USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA, [2]University of Nebraska-Lincoln, Lincoln, NE, USA, [3]3USDA-ARS, U.S. Arid-Land Agricultural Research Center, Maricopa, AZ, USA

The seed oils of the tung tree (*Vernicia* sp.) are the best naturally-occuring drying oils known to man. Many genes from the tung oil biosynthetic pathway have been cloned in our laboratories in recent years. The key enzyme is FADX,

the diverged FAD2-like enzyme that catalyzes the formation of α-eleostearic acid from linoleic acid. Preliminary analysis of transgenic *A. thaliana* plants expressing various *FADX* genes showed that tung *FADX*, driven by the French bean phaseolin promoter, produced approximately half as much eleostearic acid as was observed in plants expressing the *McFADX* gene from bitter gourd (*Momordica charantia*)*. *McFADX* was expressed behind the soybean beta-conglycinin promoter. It is possible that differences in promoter strength and timing during *A. thaliana* seed development may have affected the outcomes. Determination of optimal expression conditions for tung FADX will be essential for a successful engineering strategy to produce tung-like drying oils in transgenic systems. Optimal conditions for FADX expression were determined by comparing a variety of variables, including three different selection methods, three different epitope tags, and five different seed-specific promoters. The results of these studies and a discussion of other factors that may affect transgenic drying oil production are presented here.Cahoon, E.B., Dietrich, C.R., Meyer, K., Damude, H.G., Dyer, J.M., and Kinney, A.J. (2006) Conjugated fatty acids accumulate to high levels in phospholipids of metabolically engineered soybean and *Arabidopsis* seeds. *Phytochem*. 67: 1166?1176.

Alkylglycerol Discrimination in Lipase–catalyzed Ethanolysys of Shark Liver Oil.
L. Vazquez, O. Fernandez, G. Reglero, C. Torres, Instituto de Investigación en Ciencias de la Alimentación (CIAL), 28049 Madrid Spain

Lipase–catalyzed ethanolysis of squalene free shark liver oil has been investigated. The mentioned shark liver oil was mainly comprised of diacyl glycerol ether and triacylglycerols. In order to test discrimination against alkylglycerol, five different lipases were compared. The ratio oil to ethanol and the lipase stability in three consecutive trials of ethanolysis reaction were also evaluated. Surprisingly, Lipase from Pseudomonas stutzeri was the fastest biocatalyst among all assayed although poor discrimination against alkylglycerol was attained. In this study, the best results in terms of selectivity and stability were obtained with immobilized lipase from Candida antarctica (Novozym 435). Ethanolysis reaction in the presence of Novozym 435 produced total disappearance of triacylglycerol and a final reaction mixture comprised of diacyl glycerol ethers and monoacylglycerol ethers.

Production of New Derivatives from Cariophylene Oxide, Humulene Epoxide and Epoxidated Sesquiterpene Fraction of Copaiba Oleoresin.
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Epoxides are one group of versatile chemical–building blocks. This presentation will describe the production of new chemical derivatives from cariophylene oxide, humulene epoxide and the epoxidized sesquiterpene fraction of copaiba oleoresin.The epoxidation of sesquiterpene fractions of copaiba oleoresin, as well as the epoxidation of beta–trans–cariophylene and alpha–humulene standards was done by conventional chemical and chemo–enzymatic processes, using the enzymatic promiscuity concept. The enzymatic promiscuity concept involves the possibility that one active site of an enzyme can catalyze several different chemical reactions. 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 of copaiba oleoresin in medicine described in the literature such as analgesic, cicatrizant, bacteriocide, anti–helminthes, anti–inflammatory, anti–cancer and gastro–protector. The sesquiterpene fraction contains a mixture of beta–trans–cariophylene (majority), alpha–copaene and alpha–humulene. The resin contains the diterpene acids as copalic and hardwickiic.

Isolation and Partial Characterization of Phytosterols from Mixed Rapeseed and Soybean Deodorizer Oil Distillates (DOD).
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Ethanolysis of DOD catalyzed by Lipozyme 435 converted phytosteryl esters to free phytosterols and fatty acid ethyl ester (FAEE). The FAEE was removed by vacuum distillation and the residual ethanolysed products were subjected for phytosterols isolation using different washing–crystallization systems. The highest phytosterol purity (97.3%) with a yield of 30.7% was found in the extracts prepared by washing with hexane, acetone:ethanol (4:1,v/v) and crystallization with acetone:ethanol (4:1,v/v). The FTIR spectra and the comparable Rf with cholesterol in TLC plate

suggested the predominant of phytosterols composed in all extracts. The melting point of all extracts were found in the ranges of 130–141°C. Phytosterols isolated by washing with hexane, acetone:ethanol (4:1,v/v) and crystallization with acetone:ethanol (4:1,v/v) exhibited the sharp melting peak between 138.5–140.6°C. As a consequence, washing with hexane, acetone:ethanol (4:1,v/v) and crystallization with acetone:ethanol (4:1,v/v) was an effective means for recovery of free phytosterols from DOD ethanolysed products.

Modeling and Optimization of Lipase–catalyzed Synthesis of Beneficial Wax Esters Containing Conjugated Linoleic Acid by Response Surface Methodology.

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The objectives of this study were to model the lipase-catalyzed esterification of polycosanol with conjugated linoleic acid to produce health-beneficial wax esters and to optimize the reaction conditions, using the principle of response surface methodology. Of the seven different kinds of lipases tested, Novozym 435 was the best effective biocatalyst for the reaction. The reaction factors investigated were temperature (Te = 35–65°C), enzyme amount (En= 1–5% of total substrates), and reaction time (t = 10–50 min). Well–fitting quadratic polynomial regression model for degree of esterification (DE) was established after regression analysis with backward elimination and was verified by a chi-square test. All factors investigated positively affected DE, with En having the greatest effect. The quadratic terms of En and t showed negative effects on DE, but that of Te had no effect on DE. The interaction terms of Te positively affected DE. Optimal reaction conditions established were: Te, 62.9–65.0°C; En, 4.9–5.0%; t, 40 min and DE was 95.7–96.4 mol% under these conditions.

Optimization of Lipase–Catalyzed Production of Symmetric Monounsaturated Triacylglycerols in a Packed Bed Reactor by Response Surface Methodology.

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The aim of this study was to optimize the lipase–catalyzed transesterification of high oleic sunflower oil (A) with a mixture of palmitic and stearic acid ethyl esters (B) to produce symmetric monounsaturated triacylglycerols (SMUT). The reaction was performed in a mini–scale packed bed reactor, using Lipozyme RM IM as the biocatalyst. Response surface methodology was used to optimize the reaction conditions. The independent variables were temperature (Te = 40–60°C), residence time (RT = 15–80 min), substrate molar ratio (SR = 3–9, B/A), and water content (WC = 300–1000 mg/kg). Response variables were contents of total SMUT and diacylglycerols in the reaction products and the contents of palmitic and stearic acids at the *sn*–2 position of triacylglycerols in the products. Optimal reaction conditions were: Te, 48°C; RT, 41 min; SR, 9.0; WC, 300 mg/kg, for maximizing the total SMUT content (52.8%) and for minimizing the contents of diacylglycerols and palmitic and stearic acids at the *sn*–2 position. To make the reaction cost–effective, the values of Te, RT and SR were minimized to 42°C, 33 min, and 5.6, respectively, and the maximum content of total SMUT was 41.2% under these conditions.

Optimization of Biosynthesis of the Flavor Precursors Linoleic Acid Hydroperoxides, Using Selected Sources of Linoleic Acid.

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This research was aimed at the optimization of biosynthesis by soybean lipoxygenase of linoleic acid hydroperoxides (HPODs), considered as flavor precursors, using selected sources of linoleic acid (LA). The investigated sources included pure (100%) and commercial (67%) LAs as well as safflower oil (SO) (77.7% trilinolein) and its hydrolyzed product. The optimization of the biosynthesis of HPODs was carried out using the commercial LA as substrate. The highest bioconversion yield (73.4%) of LA into HPODs was obtained with the use of 4 mM LA, 0.025 mg solid enzyme/mL, 90 min reaction time and 0.5% Tween–20 as emulsifier. The results also showed that, using 0.4% Tween–

20 and 3 out of 6 scale stirring speed, the bioconversion of pure and commercial LAs resulted in a similar HPODs yield of 69.0%, whereas that of the hydrolyzed SO, obtained by lipase, was 58.0%. When SO was used as the LA source, only trace amounts of HPODs were obtained. The effect of LA source on the ratio of the biosynthesized HPODs isomers was also investigated. Using 3.5 out of 6 scale–stirring speed and 0.2% Tween–20, the HPODs bioconversion yield was 85.9 and 74.0%, for the commercial LA and the hydrolyzed SO, respectively.

Validation and Detection of QTL for Soybean Isoflavones.

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Soybean isoflavones have gained considerable interest in recent years as a potential benefit to human health. Analytical measurement methods for soybean isoflavones can be time consuming and costly. Consequently, QTL detection for marker assisted breeding is being examined for its potential for genetic gains. This study sought to detect QTL for soybean isoflavones in three different maturity tests (early, mid, and late) in a population of 274 RILs derived from parental lines Essex and Williams 82. The field tests were grown in an RCBD replicated three times in three environments in 2009 (Knoxville, TN; Harrisburg, IL; and Fayetteville, AR). The population was genotyped with 1,536 SNP markers, of which 480 were polymorphic. Phenotypic data was collected with NIR spectroscopy. Each maturity test, containing 91 or 92 RILS, was analyzed separately for QTL. In total, 7 QTL for genistein (chromosomes 5, 6, 9, 9, 13, 17, and 19), 5 QTL for daidzein (chromosomes 5, 6, 9, 13, and 19), 3 QTL for glycitein (chromosomes 6, 9, and 20), and 6 QTL for total isoflavones (chromosomes 5, 5, 6, 9, 13, and 19) were detected. The detection of these QTL could potentially lead to genetic gains for soybean isoflavones.