

# 2020 AOCS Annual Meeting & Expo Biotechnology Abstracts

## 2020 AOCS Annual Meeting & Expo Biotechnology Abstracts

June 29 to July 3, 2020

Hosted online by the American Oil Chemists' Society (AOCS)

For more information, please visit <https://annualmeeting.aocs.org>.

Presentations dated Friday, January 1, 2021, were provided on-demand.

### Biotechnology

Tuesday, June 30, 2020

Session Time: 10:25 AM - 11:20 AM

Presentation Time: 10:25 AM - 10:30 AM

Track: Biotechnology

#### **Introduction: Oleochemicals - Biocatalysis II**

Co-Chair: Jun Ogawa, PhD - Kyoto University

Co-Chair: Lu-Kwang Ju - The University of Akron

This session emphasizes the introduction of new starting materials and products that are oils or oil-related materials. Oleochemicals are important materials for modern society; their properties and production processes are closely related to the progress of biocatalysis, which is a key tool for realizing the UN's Sustainable Development Goals. Papers presenting novel bioprocesses for producing oleochemicals by biocatalysis, e.g., using free or immobilized cells or enzymes for fermentation and/or microbial conversion in aqueous or non-aqueous systems, are welcome. These methodologies create environmentally friendly products and processes for the future of the oleochemical industry.

Tuesday, June 30, 2020

Session Time: 10:25 AM - 11:20 AM

Presentation Time: 11:20 AM - 11:20 AM

Track: Biotechnology

#### **(3874) Bioprocess Development for Novel Functional Lipid Production: Screening and Analysis of Microbial Metabolisms and Enzymes**

Presenting Author: Jun Ogawa, PhD - Kyoto University

Microorganisms are promising as producers of functional lipids such as polyunsaturated fatty acids (PUFAs) and as catalysts transforming them into various molecular species with novel

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bioactivities. Filamentous fungus *Mortierella alpina* 1S-4 produced triacylglycerols rich in arachidonic acid. Mutants defective in desaturases and elongases accumulated triacylglycerols rich in unique PUFAs, i.e., dihomog- $\gamma$ -linolenic acid, Mead acid, and n-1, n-4 and n-7 PUFAs. Some mutants produced lipids extracellularly. Molecular breeding of the strain resulted in the production of triacylglycerols rich in n-3 ( $\omega$ 3) PUFA including EPA under ordinary temperature. DHA and  $\omega$ 3-DPA rich triacylglycerol was produced by newly isolated *Aurantiochytrium* sp. T7. Enzymatic oxygenation generates bioactive PUFA derivatives such as prostaglandins. One of the prostaglandins, PGF<sub>2 $\alpha$</sub> , is generated from arachidonic acid via the cyclooxygenase (COX) reaction. COX gene from *Gracilaria vermiculophylla* was introduced into *M. alpina*. The transformant was capable to produce PGF<sub>2 $\alpha$</sub>  extracellularly. Enzymatic epoxidation of EPA by bacterial P450 monooxygenase was applied to produce anti-inflammatory derivatives such as 17,18-epoxy-eicosatetraenoic acid. Novel PUFA metabolism was found in gut bacteria. Through the metabolism, hydroxy, oxo, enone, and conjugated fatty acids were produced by the enzyme system consisting of hydratase, dehydrogenase, isomerase, and enone reductase. Enzymatic method to prepare these unique metabolites was established. The existence of these metabolites in host tissues depending on gut bacteria was revealed. The metabolites showed unique physiological activities. These results suggested that the dietary fatty acid metabolites by gut microbiota can influence the health of the host, and might have potentials as novel functional foods and pharmaceuticals.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology

### **(3559) Synthesis of Palmitoleic Acid-enriched Triglyceride from Macadamia Nut Oil Using Immobilized Lipase Under Vacuum**

Presenting Author: DongChan Oh

Palmitoleic acid enriched triacylglycerol was efficiently synthesized from palmitoleic acid-enriched fatty acid and glycerol via immobilized lipase-catalyzed esterification. The palmitoleic acid enriched fatty acid was prepared by urea complexation from fatty acids of macadamia nut oil. A low cost lipase (Eversa@ Transform 2.0, Novozymes), which is developed for production of biodiesel, was employed for immobilization with a macroporous hydrophobic carrier (Commercial name; Lewatit VP OC 1600). The effects of several parameters, such as temperature, enzyme loading, and vacuum for preparation of palmitoleic acid enriched-triglyceride were studied. The conversion increased up to 60 oC as temperature increased, but no significant differences between conversions obtained at 60 oC and 70 oC were observed. Meanwhile, the conversion decreased markedly when temperature was further increased to 80 oC. Consequently, optimal temperature, enzyme loading, and vacuum were 60 oC, 12.5% (based on the total weight of substrate), and 100 torr, respectively. Maximum conversion of ca. 87% was obtained after 12 h under these optimal conditions.

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Friday, January 1, 2021

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Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology

### **(3557) Preparation of High Purity Stearidonic Acid by Enzymatic Method Combined with Preparative Hplc**

Presenting Author: In-Hwan Kim, PhD - Korea University

Stearidonic acid (SDA), an n-3 polyunsaturated fatty acid (PUFA), can be obtained from plant origin oils and it can be a good source of PUFA for vegetarians. SDA can be easily converted to longer PUFA such as docosahexaenoic acid and eicosapentaenoic acid. Highly purified stearidonic acid (SDA) was prepared successfully from echium oil via an enzymatic method combined with preparative high performance liquid chromatography. In the 1st step, SDA enrichment was accomplished using *Candida rugosa* lipase and 39.5% of SDA was obtained in the fatty acid fraction. Subsequently, the 1st reaction mixture was used for the 2nd enzymatic esterification without any separation process. The 2nd esterification was conducted for further SDA enrichment in a packed-bed reactor using Lipozyme RM IM from *Rhizomucor miehei* and the SDA content increased in a very short residence time. Ethanol was selected as an appropriate alcohol to react as an acyl receptor, and the other conditions for SDA enrichment were optimized at 20 °C of temperature, and 1:4 of molar ratio (i.e., fatty acid to ethanol). Under these conditions, 51.6% of SDA was obtained in the fatty acid fraction after a residence time of 15 min. Finally, highly purified SDA (purity, >99%) was obtained by prep-HPLC using the SDA-rich fraction obtained from the two-step lipase-catalyzed esterification.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology

### **(3845) Immobilized Phospholipase A<sub>1</sub>-catalyzed Preparation of L- $\alpha$ -glycerylphosphorylcholine from Soy Phosphatidylcholine**

Presenting Author: Byung Hee Kim, PhD - Sookmyung Women's University

L- $\alpha$ -Glycerylphosphorylcholine (L- $\alpha$ -GPC) is a derivative of phosphatidylcholine where both the acyl groups are removed by hydrolysis. L- $\alpha$ -GPC enhances cholinergic neurotransmission by directly elevating acetylcholine levels in the hippocampus, thereby used as a cognitive enhancer for treating patients with stroke or dementia, including Alzheimer's disease. The aim of this study was to prepare L- $\alpha$ -GPC using immobilized phospholipase A<sub>1</sub>-catalyzed hydrolysis of soy phosphatidylcholine, followed by a purification step using phase-separation of biphasic media. An aqueous solution of commercial phospholipase A<sub>1</sub> was immobilized on a hydrophobic support material. Soy phosphatidylcholine was completely hydrolyzed to L- $\alpha$ -GPC using the immobilized phospholipase A<sub>1</sub> as the biocatalyst under optimal conditions that was determined

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by response surface methodology. Phosphocholine, which was generated as a by-product via free phospholipase A<sub>1</sub>-catalyzed hydrolysis of phosphatidylcholine was little formed during the reaction. Finally, high-purity L- $\alpha$ -GPC was obtained from the reaction products after phase separation of the media without further purification step.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology

### **(3859) Development and Application of Enzymatically Structured Lipids As Alternative Cream Fats.**

Presenting Author: Jung-Ah Shin - Gangneung-Wonju National University

Palm mid-fraction (PMF) is a widely used source for the confectionary industry. It has a high content of symmetrical POP triacylglycerol (TAG) molecule. Acyl migration is the reversible shifting of the fatty acids which compose TAG between the sn-1,3 and sn-2 positions. Lipids that are restructured after acyl migration have different physicochemical characteristics than they did before the reaction. The aim of this study was to prepare an alternative fat blend with low saturated fatty acid content but also solid fat content at 25–37 °C that is similar to hydrogenated coconut oil (HCO). In this study, PMF was converted to asymmetric PPO (APMF) via acyl migration. After solvent fractionation, the liquid phase of acyl migrated PMF (APMF-L) was obtained and blended with HCO (50:50, w/w). Finally, an alternative fat blend which had reduced saturated fatty acid content was produced with melting behavior similar to that of HCO. The alternative fat blend in this study could be used as a raw material for non-dairy cream with low saturated fat content. A pH-stat titration system has been used to evaluate lipolysis rates of lipids by in vitro digestion. In this study, a physical oil blend (PHY) and an interesterified oil (IO) were prepared from soybean oil (SBO) and pomegranate oil (PGO). The effect of lipolysis on TAG molecules with different compositions was studied using a pH-stat digestion model. The lowest lipolysis rate from the PGO emulsion seems to be mainly due to the TAG species that are comprised of CL<sub>n</sub>-CL<sub>n</sub>-CL<sub>n</sub> TAG molecule (CL<sub>n</sub>, conjugated linolenic acid).

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Session Time: 1:00 AM - 2:00 AM

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Track: Biotechnology

### **(3880) Deacidification Method for the Synthesis of Triacylglycerols Containing Hydroxy Fatty Acids As Constituent Fatty Acids**

Presenting Author: Shigenobu Kishino - Kyoto University

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[Introduction] 10-Hydroxy-cis-12-octadecenoic acid (HYA) produced via saturation metabolism of linoleic acid to oleic acid by lactic acid bacteria has an intestinal barrier protecting function and an effect of suppressing triacylglycerol (TG) accumulation in the liver. There is a possibility that HYA is useful for preventing obesity. Since free form fatty acids have pungent tastes, it is desirable to produce HYA in the TG form. We succeeded to produce TG containing HYA as a constituent fatty acid using lipase applicable for food manufacturing process, while free fatty acids in the products reached about 60 % of the total lipids. In this study, we report deacidification method for the free fatty acid-containing products. [Results and discussion] We examined for following two methods for the deacidification. I. We attempted to adsorb free fatty acids to an anion exchange resin with aqueous ethanol solutions of various concentrations. Evaluation of free fatty acid contents revealed that less than 1 % of free fatty acid content was achieved when 90 % ethanol aqueous solution was used. II. Taking advantage of the difference in solubility of lipids in aqueous ethanol solutions, separation of TG and free fatty acids was attempted by using a PTFE membrane. Samples were added to aqueous ethanol solutions of various concentrations, and the solutions were stirred to form o/w emulsions, and then filtered through a PTFE membrane. Evaluation of free fatty acid contents revealed that less than 2 % of free fatty acid content was achieved.

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Session Time: 1:00 AM - 2:00 AM

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Track: Biotechnology

### **(3958) Gas-to-lipids bioprocessing for carbon recycling**

Presenting Author: Tsunehiro Aki, PhD - Hiroshima University

Reducing greenhouse gas emissions has become a pressing issue worldwide. We have previously constructed a system that simultaneously achieves carbon fixation and useful lipid production by a two-stage fermentation system with different microorganisms. These studies have provided hints for developing new uses of the oleaginous microorganism, *Aurantiochytrium* sp., showing high assimilability of acetic acid with combination to the acetogen, *Acetobacterium woodii*, which releases acetic acid when fixing carbon dioxide. Whole culture of the acetogen can be supplied for lipid fermentation without component fractionation. The metabolome and transcriptome analyses have been conducted to determine target factors to edit their genes for improvement of lipid productivity by this integrated gas-to-lipids bioprocessing.

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Session Time: 1:00 AM - 2:00 AM

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Track: Biotechnology

### **(4224) Sustainable Lipid Feedstock for Food Security**

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Presenting Author: Ghulam Kadir Ahmad Parveez, PhD - Malaysian Palm Oil Board

Agricultural industry must increase food production by more than 70% come 2050 when population is predicted to touch 9 billion. Various sources of food ranging from carbohydrates to proteins to oils and fats are expected to double or even triple to meet demands. An evolving population with environment-centric mindset pushing for renewable energy source further adds to the need for more oils. The imbalance in production and demand if not address appropriately will soon grow to become a threat to global food security. This is further exacerbated by climate change. Oil palm is known to be the most productive oil-bearing crop; therefore, it is without a doubt that it would be the most sustainable lipid source to meet the world's growing demand. In spite of its naturally highly productive nature, the industry continues to be vilified by those who claimed it to be the main cause of deforestation and a source of unhealthy oil. Taking it all in its stride, the palm oil industry has put in place strategies stretching from upstream to downstream to mitigate issues that may threaten its posterity. From developing new planting materials to improving processes through technology adoption while ensuring prosperity for all, the industry will keep innovating and re-inventing itself to survive and to safeguard the future of food. Hence, if palm oil is unavoidable, can it be made more sustainable?

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology

### **(3882) Improvement of Free Fatty Acid Productivity by Controlling Carbon Metabolism in Cyanobacteria.**

Presenting Author: Akihito Kawahara - Kao corporation

Production of biofuels from microalgae has been developed during the recent decades. In particular, cyanobacteria have already been established as promising hosts for production of various fatty acid derivatives. The cyAbrB2 transcriptional regulator in the cyanobacterium *Synechocystis* sp. PCC 6803 is involved in coordination of carbon and nitrogen metabolism, and its deletion causes high accumulation of glycogen granules. In this study, to establish metabolic modification technology that promotes the utilization of photosynthetic-fixed carbon for fatty acid biosynthesis, we evaluated the effects of deletion of cyAbrB2 ( $\Delta cyabrB2$ ) on free fatty acid (FFA) productivity. The  $\Delta cyabrB2$ -background FFA producing strain showed more than two-folds higher FFA productivity compared with WT-background strain, without expense of the accumulated glycogen. Next, to redirect the excess carbon stored as glycogen to FFA synthetic pathway, we constructed the conditional double deletion mutant of cyAbrB2 and the glycogen synthetic pathway. Metabolome analysis and electron microscopic observation revealed that the double deletion causes increase of organic acids in the tricarboxylic acid cycle, amino acids and storage compounds such as polyhydroxybutyrate (PHB). Furthermore, we succeeded to increase FFA productivity of the double deletion mutant by the prevention of accumulation of PHB. Here, we propose the triple deletion of cyAbrB2, glycogen- and PHB-synthetic pathways as a novel

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technology for enhancement of FFA productivity by controlling the carbon metabolic flux of *Synechocystis*.

Tuesday, June 30, 2020

Session Time: 10:25 AM - 11:20 AM

Presentation Time: 11:20 AM - 11:20 AM

Track: Biotechnology@@@Analytical

### **(3823) FA Distribution in Infant Formula Lipids Analysed by Joint JOCS/AOCS Official Method Ch 3a-19**

Presenting Author: Yomi Watanabe, PhD - Osaka Research Institute of Industrial Science & Technology

Joint JOCS/AOCS Official Method Ch 3a-19 is a newly adopted method for the positional analysis of the fatty acid (FA) distribution of fats and oils. As the catalyst, it uses *Candida antarctica* lipase, which is active on a broad spectrum of FA. Thus, in contrast to the conventional method using pork pancreas lipase, the method is applicable to fats and oils containing short- chain and polyunsaturated FAs such as milk fat, fish, and algal oils. When the method was applied to milk fat, it was determined that short- chain fatty acids are located mainly at *sn*-1(3) position of milk fat (doi:10.5650/JOS.ess15008). Here, we report the application of the method to processed food containing milk fat. For the first time, the method was applied to the fats of infant formula. Infant formula lipids were extracted using the Röse-Gottlieb method from six commercial infant formulas; recoveries of 85-95% were obtained. The extracted fats were composed mainly of triacylglycerols (TAG) (~98%) and the major FAs detected were 16:0, 18:0, 18:1, 18:2. Using Official Method Ch 3a-19, we determined that 16:0 FA is predominantly located at the *sn*-2 position of infant formula TAGs. whereas 18:1 FA is predominantly located at the *sn*-1(3) positions of infant formula TAGs. In addition, we found that the medium-chain FAs, 6:0, 8:0, 10:0, tended to be located at the *sn*-1(3) positions of infant formula TAGs. We confirmed that the ingredient labels of all six infant formulas accurately indicated the FA compositions and distributions of the infant formulas. The content of PUFAs detected in all the fats of the infant formulas we analyzed was less than <1%. In conclusion, by combining the Röse-Gottlieb extraction method with Official Method Ch 3a-19, the FA distribution of the lipids of commercial infant formulas was successfully analyzed.

Tuesday, June 30, 2020

Session Time: 11:20 AM - 1:00 PM

Presentation Time: 12:10 PM - 12:10 PM

Track: Biotechnology@@@Analytical

### **(3976) Gondoic acid, a novel oleochemical feedstock from seed oils.**

Presenting Author: Mark A. Smith, PhD - Agriculture and Agri-Food Canada

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Gondoic acid (*cis*-11-eicosenoic acid, 20:1 $\Delta$ 11) is an uncommon 20-carbon fatty acid that is a component of the seed oil of a small number of plant species. This fatty acid is a potential renewable feedstock for the polymer industry representing a source of C12 monomers through metathesis, or C11 monomers through reductive ozonolysis. *Camelina sativa* is the only oilseed crop that produces significant levels of gondoic acid, but at 13-15% of total seed fatty acids, camelina seed oil is not a commercially viable source of this fatty acid. To identify new sources of gondoic acid, a survey of seed oil composition by GC and GC/MS was conducted for diverse plant species. Oils containing up to 46% gondoic acid were identified, often with additional very-long-chain fatty acids present. Further investigation of the oils by NMR, TLC and MALDI-TOF MS revealed novel storage lipids, including acetyl-TAG and cyanolipids, and oils with unusual fatty acid positional distribution. Oils containing gondoic acid from members of the Ranunculaceae were unusual in that the fatty acid was not accompanied by significant amounts of erucic acid (*cis*-13-docosenoic acid, 22:1 $\Delta$ 13). Developing seeds from two species were collected and transcriptome analysis enabled the identification of a novel pathway for gondoic acid biosynthesis. cDNAs encoding the enzymes for fatty acid elongation and incorporation into TAG were cloned and ectopically expressed in *Camelina*. Prototype plants showed a modest increase in seed gondoic acid levels. Characterization of these plants is suggesting new ways to increase gondoic acid levels in camelina seed oil.

Tuesday, June 30, 2020

Session Time: 10:25 AM - 11:20 AM

Presentation Time: 10:30 AM - 10:55 AM

Track: Biotechnology@@@Edible Applications Technology

### **(3497) Effect of Calcium Concentration on Lipid Digestion and $\beta$ -carotene Bioaccessibility Using INFOGEST Protocol**

Presenting Author: Yunbing Tan, MA - University of Massachusetts

Recently, a standardized in vitro digestion model (“INFOGEST method”) has been developed to evaluate the gastrointestinal fate of foods. Under fed state conditions, the calcium level used in this model is fixed and relatively low: 0.525 mM. In practice, the calcium concentration in the human gut depends on the nature of the food consumed and may vary from person-to-person. For this reason, we examined the impact of calcium concentration on the gastrointestinal fate of a model nutraceutical delivery system. The effect of calcium level (0.525-10 mM) on lipid digestion and  $\beta$ -carotene bioaccessibility in corn oil-in-water nanoemulsions was investigated using the INFOGEST method. At all calcium levels, the lipids were fully digested, but this could only be established by carrying out a back titration (to pH 9) at the end of the small intestine phase. Conversely, the bioaccessibility of  $\beta$ -carotene decreased with increasing calcium levels: from 65.5% at 0.525 mM Ca<sup>2+</sup> to 23.7% at 10 mM Ca<sup>2+</sup>. This effect was attributed to the ability of the calcium ions to precipitate the  $\beta$ -carotene-loaded mixed micelles by forming insoluble calcium soaps. Our results show that the bioaccessibility of hydrophobic carotenoids

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measured using the INFOGEST method is highly dependent on the calcium levels employed, which may have important consequences for certain calcium-rich foods.

Tuesday, June 30, 2020

Session Time: 11:20 AM - 1:00 PM

Presentation Time: 12:35 PM - 12:35 PM

Track: Biotechnology@@@Edible Applications Technology

### **(3820) Efficient Production of Structured Lipids by Lipase Reaction**

Presenting Author: Hidenori Konishi, Nisshin OilliO - Central Research Laboratory

Efficient Production of Structured Lipids by Lipase Reaction We can make functional structure lipids by using lipase catalyzed interesterification because lipase has position specificity of esterification. It can be conducted at normal temperature and pressure, so deterioration of reactants can be reduced and side reaction can be controlled. On the other hand, lipase catalyzed interesterification has a lot of problems. One of the major problems is its high cost. To reduce cost, maintaining stability of lipases is very important. For the purpose of overcoming this problem, we tried to suppress the decreasing lipase stability by optimization of reaction system. Specifically, we adjusted reaction conditions and reaction mode (batch or continuous) thereby establishing a stable reaction system. From the above studies, we could maintain stability of the lipase activity and achieve the stable production system. This reaction system can be applied to interesterification not only between triacylglycerol (TAG) but also between TAG and fatty acids or ester. We introduce these examples of practical application in this presentation.

Thursday, July 2, 2020

Session Time: 10:25 AM - 12:35 PM

Presentation Time: 10:30 AM - 10:55 AM

Track: Biotechnology@@@Health and Nutrition

### **(3791) Bio-accessibility of Lipids from Chlorella Vulgaris**

Presenting Author: Fabiola Dionisi, PhD - Societe' des Produits Nestlé - Nestlé Research

Microalgae are emerging as alternative, vegan source of polyunsaturated fatty acids, protein and micronutrients. Microalgae producing  $\omega$ 3-PUFAs can be grown heterotrophically without light on low-cost substrates. Chlorella spp. contains up to 27% ALA in the lipid fraction. Microalgae lipids are usually used upon extraction. For food supplements or ingredients, whole biomass can be also consumed as such. For example, dried biomasses of Chlorella spp. are commercially available as source of protein and healthy lipids. However, bioaccessibility of lipids, as well as other nutrients, in the whole microalgae cell has not been extensively studied. Bioaccessibility is strictly related to cell wall composition and structure. The cell wall consists of a matrix of

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glycoprotein and polysaccharides resistant to digestion and whose composition depends on species and growth stage. Downstream treatments could be used to enhance the nutrient bioaccessibility. Enzymatic hydrolysis with chitinase, lysozyme, pectinase, sulfatase,  $\beta$ -glucuronidase, and laminarinase was reported to have a positive effect on cell wall degradation. Moreover, enzymatic treatment of the cell wall has major advantages over mechanical treatments, such as low energy requirements, and mild and pollutant free processing conditions (Baudeflet et al., 2017). Also, enzymatic treatments do not cause a temperature stress, preserving the quality of  $\omega$ 3-PUFAs. The fatty acid profile of five commercial *Chlorella* spp. biomasses was evaluated and compared to the *C. vulgaris* grown in our lab. All biomasses showed a good (5:1) to ideal (1:1)  $\omega$ 6: $\omega$ 3 ratio. Lipid bioaccessibility was measured through INFOGEST protocol, resulting in less than 7% for commercial biomasses. Regarding the effect of growth phase, this was studied in *C. vulgaris* (CCALA 256) grown in heterotrophic controlled conditions in a 16 L fermenter, for seven days, reaching concentrations of  $7.1 \pm 0.3$  g/L. ALA accounted for  $20\% \pm 2\%$  in *C. vulgaris* total fatty acids. Results showed that lipids were more bioaccessible in stationary compared to exponential phase. To increase the bioaccessibility, a treatment with chitinase and lysozyme on *C. vulgaris* biomass was performed. A 10% increase in both protein and lipid bioaccessibility was reached. This research shows how harvesting time influence lipid productivity and bioaccessibility and that downstream treatments are needed to increase the bioaccessibility of nutrients in microalgae biomasses.

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Track: Biotechnology@@@Health and Nutrition

### **(3589) Valorization of Crude Olive Pomace Oil: Lipase-Catalyzed Production of Dietetic Structured Lipids, Emulsifiers and Biodiesel**

Presenting Author: Suzana Ferreira-Dias - Universidade de Lisboa, Instituto Superior de Agronomia

Olive oil is the major oil produced and consumed in the Mediterranean countries. After olive oil extraction by mechanical processes, the residual oil (c.a. 3-4 %) present in the olive pomace (olive pomace oil) can be solvent extracted and used for edible purposes after refining. This study aimed at the valorization of the crude olive pomace oil for the synthesis of value-added compounds (low-calorie structured lipids and monoacylglycerols, MAG) and biodiesel, following the biorefinery concept and circular economy strategies. Low-calorie triacylglycerols (TAG), known as MLM, are structured TAG containing a long-chain fatty acid (L) at the position sn-2 and medium-chain fatty acids (M), at the external positions of TAG. MLM are important to control obesity, since they present a caloric value of 5 kcal/g. Crude olive pomace oil was used to produce MLM by (i) acidolysis with medium-chain fatty acids, namely caprylic (C8:0) or capric (C10:0) acids; or (ii) interesterification with ethyl octanoate (C8EE), or ethyl decanoate (C10EE), in solvent-free media. Immobilized sn-1,3 regioselective lipases (Lipozyme RMIM and Lipozyme TLIM, Novozymes, Denmark) were used as biocatalysts. Apparent

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equilibrium was attained after 7h reaction. MLM yields showed to be independent from the initial acidity of the crude oil. Lipozyme RM IM presented high operational stability during 13 consecutive 7h-batches. Crude olive pomace oil was also used for biodiesel (fatty acid methyl esters/FAME) production by esterification/transesterification with methanol, catalyzed by immobilized sn-1,3 selective lipases. Thus, FAME and MAG (emulsifiers) instead of glycerol, were obtained overcoming the problem of surplus of low-quality glycerol.

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Track: Biotechnology@@@Health and Nutrition

### **(3858) Preparation and Bio-functions of Polyunsaturated Phosphatidylglycerol**

Presenting Author: Masashi Hosokawa, PhD - Hokkaido University

n-3 Polyunsaturated fatty acids enriched phosphatidylglycerol (n-3 PUFA-PG) is expected to be a highly functional phospholipid (PL), which has many physiological functions and shows excellent liposome forming activity. However, naturally occurring n-3 PUFA-PG is low in content, resulting in a scarcity of industrial bio-resources. We established a n-3 PUFA-PG preparation method using marine phospholipids such as salmon roe lipids via phospholipase D (PLD)-mediated transphosphatidylation. The yield of n-3 PUFA-PG from salmon roe total lipid in the aqueous system without organic solvent reached 96.4 mol%, following a 24 h reaction. Synthesized n-3 PUFA-PG significantly reduced the total and non-HDL cholesterol in the serum of diabetic/obese KK-Ay mice. In the mice fed n-3 PUFA-PG, but not n-3 PUFA-TAG, hepatic lipid content was markedly alleviated depending on the neutral lipid reduction compared with the SoyPC-fed mice. Furthermore, n-3 PUFA-PG down-regulated mRNA expression of pro-inflammatory mediators including IL-6, IL-1 $\beta$ , iNOS and COX-2 in LPS-stimulated RAW264.7 cells. The anti-inflammatory effects were expressed through Nrf-2 activation. These results indicate that n-3 PUFA-PG prepared from marine PL by PLD-mediated transphosphatidylation is the highly functional lipid.

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Track: Biotechnology@@@Health and Nutrition

### **(3827) Innovation in Oilseed Crops**

Presenting Author: Surinder P. Singh - CSIRO

Over the last two decades, advances in understanding of the biochemical, cellular, and molecular mechanisms of plant oil biosynthesis, coupled with the cloning of many of the genes involved in this process, have facilitated the production of designer plant oils with improved nutritional

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benefits and enhanced functional properties. In this talk I will describe the development of a canola crop with DHA and EPA in its seed oil. The increasing demand for these oils provides an urgent need for an alternative, safe, and sustainable source of DHA and EPA. The development of a DHA-rich canola crop is a triumph in plant metabolic engineering, in both the complexity of the biosynthetic pathways that have been transferred to higher plants, and the number of genes that have been introduced to encode these pathways. The DHA-rich canola crop has been approved by regulators for cultivation in Australia and USA and will enter commercial production from 2020 onwards. I will also discuss the development of safflower containing 93% oleic acid in the seed oil which should enable greater industrial application of oleic acid as a chemical feedstock and also added stability for direct industrial applications, such as in lubricants and transformer fluids. This crop has been approved by the gene regulator and is expected to enter commercial production in Australia in 2020. Finally, I will talk about the engineering of seed oil-like levels in leaves of plants. This approach has the potential to revolutionise the way plant oils are produced, with palm oil-like productivity achievable if translated to high-biomass C4 crops like sorghum or miscanthus.

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Track: Biotechnology@@@Health and Nutrition

### **(4114) Interactions of polyphosphate biosynthesis with triacylglycerol biosynthesis in the model alga *Chlamydomonas reinhardtii***

Presenting Author: Yantao Li, PhD - the Institute of Marine and Environmental Technology (IMET), the University of Maryland

Microalgal lipid biosynthesis has been extensively studied, particularly under nitrogen depleted conditions where triacylglycerol (TAG) rapidly accumulates. However, our knowledge about interaction of algal TAG biosynthesis with other nutrient uptake pathways, particularly phosphate uptake and polyphosphate biosynthesis remains limited. In this study, polyphosphate biosynthesis and its link with lipid biosynthesis pathways was explored in the model alga *Chlamydomonas reinhardtii* using a mutant defective in the vacuolar transporter chaperone 1 gene (VTC1). Deletion of the VTC1 gene ( $\Delta vtc1$ ) downregulated the expression of the phosphate transporter genes and impaired phosphate uptake. As a result, the  $\Delta vtc1$  mutant accumulated little polyphosphate. By contrast, the  $\Delta vtc1$  mutant accumulated more TAG under phosphorus depleted conditions, resulting in a 50% increase in TAG content. This was accompanied by overexpression of the phospholipid: diacylglycerol acyltransferase (PDAT) but not diacylglycerol acyltransferase (DGAT) genes. Interestingly, double knockout of VTC1 and PDAT ( $\Delta vtc1\Delta PDAT$ ) increased TAG content drastically by 182.3%, suggesting compensating pathways that may contribute to TAG formation. Further analysis of the mutant revealed one of the DGAT genes, DGTT1 was responsible for enhanced TAG biosynthesis in the double knockout mutant. Impairment of polyphosphate biosynthesis also affected the fatty acid composition of TAG, promoting accumulation of mono- and polyunsaturated fatty acids at the

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expense of saturated fatty acids. These data suggest a new strategy to improve the quality and yield of algal oils through manipulation of polyphosphate biosynthesis. The biotechnological applications of DGAT manipulation for polyunsaturated fatty acids production were also discussed.

Monday, June 29, 2020

Session Time: 8:25 AM - 10:10 AM

Presentation Time: 8:30 AM - 8:55 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4193) Research and Development of Novel Pressure Sensitive Adhesives from Vegetable Oils**

Presenting Author: Kaichang Li - Oregon State University

Pressure sensitive adhesives (PSAs) are widely used in pressure sensitive labels, tapes, stamps, Post-it notes and many other products. At present, PSAs are mainly derived from petrochemicals that are not renewable and not sustainable. In this presentation, I will disclose our strategies of converting vegetable oils into superior PSAs. We discovered that different polyesters from derivatives of soybean oils such as epoxidized soybean oil could have superior PSA properties. The polyesters can be prepared from polymerization of epoxidized fatty acids such as epoxidized oleic acid, a bifunctional AB monomer containing a carboxylic acid group (A) and an epoxy group (B); polymerization of epoxidized soybean oils containing multiple epoxy groups and a dibasic acid such as dimer acids; or polymerization of epoxy resins containing two epoxy groups and a dibasic acid. Innovative methods for preparation of PSAs with superior properties have been successfully developed. The resulting PSAs have been characterized for their molecular structures, thermal stabilities, and viscoelastic properties. The peel strength, shear strength, tack, and aging stability of the resulting PSAs have also been evaluated and will be discussed in detail in this presentation. The relationships between chemical structures of derivatives of soybean oils, and PSA properties have been extensively investigated and will be present and discussed in detail.

Monday, June 29, 2020

Session Time: 10:25 AM - 12:10 PM

Presentation Time: 10:30 AM - 10:30 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3696) Lipase-catalyzed Preparation of Neopentyl Glycol Diester As a Biolubricant**

Presenting Author: Jiwon Kim - Korea University

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Neopentyl glycol diester as a biolubricant was successfully synthesized in a solvent free system with fatty acid and neopentyl glycol using an immobilized lipase as a biocatalyst. Immobilization was carried out with a liquid enzyme, Eversa transform 2.0 (from *Thermomyces lanuginose*) and Lewatit VP OC 1600, a macroporous hydrophobic resin, as a carrier. The effects of enzyme loading and temperature as a function of reaction times were explored under no vacuum condition. The maximum yield of ca. 63% was achieved at optimal temperature of 50 °C and enzyme loading of 5% (based on the total weight of the substrate) under no vacuum. However, the yield of neopentyl glycol diester increased markedly up to 97%, even though vacuum as low as 200 torr was applied. No significant differences in the yield of neopentyl glycol diester between constant vacuum condition, and combination of vacuum and no vacuum condition were observed. This combination method employed in this study is a novel strategy for synthesis of neopentyl glycol diester.

Tuesday, June 30, 2020

Session Time: 8:25 AM - 9:45 AM

Presentation Time: 8:30 AM - 8:55 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3954) Fatty Acid Hydratase for Value-added Biotransformation**

Presenting Author: Zheng Guo, PhD - Aarhus University

The synthesis of hydroxy fatty acids (HFAs) from the renewable oil feedstock by addition of water to C=C bonds have attracted great attention in recent years. Considering that selective asymmetric hydration of (non-activated) C=C bonds has been proven difficult to achieve with chemical catalysts, enzymatic catalysis by fatty acid hydratases (FAHs) present an attractive alternative route to produce value-added products in a highly regio- and enantiospecific fashion with excellent atom economy. Even though FAHs have just been investigated as a potential biocatalyst for a decade, remarkable information about FAHs in different aspects is available; however, a comprehensive review has not been archived. Herein, we summarize the research progresses on biochemical characterization, structural and mechanistic determination, enzyme engineering, as well as biotechnological application of FAHs. The current challenges and opportunities for an efficient utilization of FAHs in organic synthesis and industrial applications are critically discussed.

Tuesday, June 30, 2020

Session Time: 11:20 AM - 1:00 PM

Presentation Time: 11:20 AM - 11:20 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3626) 92% Oleic Safflower Oil: A Game Changer for the Oleochemicals Industry**

Presenting Author: Michael Honeychurch - GO Resources

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Plant-derived oils are mixtures of saturated, monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic and linolenic acids) in ratios that are less than ideal for industrial uses. Vegetable oil containing high oleic levels and minimal linoleic/linolenic levels have long been considered a desirable replacement for petroleum-based precursors in the manufacture of plastics, lubricants and cosmetics etc, due to the unique physical and chemical properties. Safflower (*Carthamus tinctorius* L.) seed produces oil that predominantly contains oleic and linoleic acids, and no linolenic acid. Traditional breeding programs have developed safflower seed with oleic acid levels in the range of 75–85%. However, like other oilseeds, the remaining linoleic acid component, at 12-18%, is undesirable for industrial use due to its increased propensity to oxidise relative to oleic acid. Therefore, it is desirable to develop a safflower seed that produces high oleic oil but contains very low linoleic content. Two genetically modified safflower events were developed by the Commonwealth Scientific Industrial Research Organisation (CSIRO) and are being commercialized by GO Resources Pty Ltd. The events contain a construct designed to down regulate two safflower fatty acid biosynthesis genes. Down regulation is achieved using RNAi technology and is targeted to the seed using a seed specific promoter. Down regulation of the two safflower genes leads to accumulation of 92% oleic acid and ~2% linoleic acid in the seed, Super High Oleic Acid Safflower Oil (SHOSO). Details of the development and commercialization of this new GM crop in Australia will be presented.

Tuesday, June 30, 2020

Session Time: 11:20 AM - 1:00 PM

Presentation Time: 11:45 AM - 12:10 PM

Track: Biotechnology@@@Industrial Oil Products

### **(4122) Chinese Violet Cress: Novel Seed Oil Biosynthesis, Storage, and Functionality**

Presenting Author: Edgar Cahoon, PhD - University of Nebraska-Lincoln

The seed oil of Chinese violet cress (*Orychophragmus violaceus*), a Brassicaceae species native to China, is enriched in novel C24 dihydroxy fatty acids, designated wuhanic acid [7,18-(OH)<sub>2</sub>-24:2Δ<sup>15,21</sup>] and nebraskanic acid [7,18-(OH)<sub>2</sub>-24:2Δ<sup>15,21</sup>]. Genetic and biochemical evidence is most consistent with the biosynthesis of these fatty acids through a pathway involving a conventional FAD2-type hydroxylase and a variation of fatty acid elongation involving extension of a 3-OH C20 intermediate prior to the completion of a complete elongation cycle. This variant pathway, referred to as “discontinuous fatty acid elongation”, is initiated by a divergent FAE1-type 3-ketoacyl-CoA synthase. In addition to their novel route of biosynthesis, wuhanic and nebraskanic acids are stored in Chinese violet cress seeds in high molecular weight triacylglycerol (TAGs) species that contain an extensive network of dihydroxy fatty acids that are cross-linked as estolides. This novel fatty acid storage results in vegetable oil with superior high-temperature lubricant properties relative to castor oil. Efforts are underway to evaluate Chinese violet cress as a new oilseed crop and to develop genetic resources for agronomic improvement of this plant.

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Thursday, July 2, 2020

Session Time: 10:25 AM - 12:35 PM

Presentation Time: 11:20 AM - 11:45 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4020) High-oleic oil of yeast as potential feedstock for biodiesel production from lignocellulosic pentoses**

Presenting Author: Mahesh B. Khot, PhD - University of Concepcion, Chile

Single cell oils (SCOs) accumulated by oleaginous yeasts have emerged as potential alternative feedstocks for biodiesel production. As microbial lipid accumulation is species and substrate specific, selection of an appropriate strain is critical. This study evaluates the biomass of newly isolated yeast *Scheffersomyces coipomoensis* CB1 for biodiesel production based on its lipid content and fatty acid profile. Yeast biomass was produced by batch fermentation in shake flasks under a high carbon/nitrogen (C/N) ratio to induce intracellular lipid accumulation using D-xylose as sole carbon source. Biomass was harvested by centrifugation, heat-dried and pulverized. Total cellular lipids were extracted as fatty acid methyl esters (FAME) by in situ transesterification and estimated gravimetrically. The chemical composition of fatty acids was characterized by GC-FID analyses. The key fuel properties – density, kinematic viscosity, cetane number, cold flow properties and oxidative stability were calculated from the fatty acid composition. Yeast strain generated 5 g/L biomass with 25 % lipid (w/w) on dry weight basis under un-optimized conditions. The yeast lipids showed the major presence of monounsaturated fatty esters (18:1,16:1) suitable for better ignition quality, oxidative stability, and cold-flow properties of the biodiesel. Analyzed fuel properties of the yeast oil were in good agreement with international biodiesel standards. The use of less toxic co-solvents or environment-friendly lipid extraction method together with solvent recovery and recycling would help improve process economics for sustainable production of biodiesel from the high-oleic yeast oil using the hemicellulosic fraction of agro-wastes.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3940) Corn-based Byproducts as Feedstocks for Polyhydroxyalkanoate Biosynthesis**

Presenting Author: Richard D. Ashby, PhD - USDA/ARS/ERRC

In addition to its nutritional value, corn provides a source of valuable intermediates for the synthesis of biofuels (e.g. ethanol, biodiesel) and other chemical compounds. Polyhydroxyalkanoates (PHA) are bacterial polyesters that are considered ‘environmentally benign’ owing to their degradation potential to CO<sub>2</sub> and water in both terrestrial and aquatic (marine) environments. Unfortunately, their cost-to-produce has limited their widespread applications but because of the mounting worldwide plastic disposal problems, these valuable

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biorenewable polymers are becoming more appealing, especially for single-use applications. Corn provides intermediates that can be utilized under fermentation conditions to produce PHA biopolymers. First, corn oil can be converted to alkyl esters through the process of transesterification to produce biodiesel with a byproduct stream (approx. 10%) of crude glycerol and second, the residual lignocellulosic biomass (corn stover - including stalks, leaves, cobs) contain fermentable sugars that can also be used to produce PHA. Both crude glycerol and corn stover hydrolysate (CSH) are relatively inexpensive byproducts of corn and as such, successful use of these materials has stimulated interest as a means of controlling the production costs of PHA and helping to improve application potential. This presentation will focus on our results using crude glycerol and CSH to produce PHA biopolymers with controllable mechanical properties.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4164) Circular Economy Driven Sustainable Biocomposites: Innovation towards Alleviating Climate Change**

Presenting Author: Amar K. Mohanty - University of Guelph

Sustainable biocomposites from renewable, recycled, and waste sources are gaining more attraction recently in the manufacturing sectors with applications ranging from light-weight automotive parts to green packaging. The whole world looks toward a low-carbon and decarbonized economy through an innovative circular economy unlike through a linear approach to mitigate the dangers of climate change. The issue of plastic waste is making headlines on a daily basis, where several countries are restricting/banning single-use plastic. Currently, the world produces around 450 million tons of plastic annually, which is expected to surpass one billion by 2050. As per a UN report, the world makes 300 metric tons/year of plastic waste which is nearly equivalent to the weight of the entire human population. It is expected that the sea will have more plastic than fish. Similarly, it is estimated that billions upon billions of tons in food waste including agro-/forestry residues and by-products and coproducts from various industrial processes are created. The philosophy of “Nothing is waste – wastes are resources” supports the concept of a circular economy. The renewable nature of bioplastics is not enough to claim them as sustainable from a commercial perspective. A group of researchers at the Bioproducts Discovery and Development Centre are working on developing cost-competitive biocomposites for uses in eco-friendly auto-parts, consumer products, and compostable packaging. This presentation will highlight the research on waste valorization and how innovation can be garnered through a unique circular approach which is made possible from University-Industry-Government collaborations.

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Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3558) Efficient Synthesis of 2-ethylhexyl Palmitate Via an Immobilized Lipase-catalyzed Esterification**

Presenting Author: Suhyeon Choi - Korea University

2-Ethylhexyl palmitate (2-EP) from 2-ethyl hexanol and palmitic acid was synthesized successfully in a solvent free system via an immobilized lipase-catalyzed esterification. A commercial lipase (Eversa@ Transform 2.0, Novozymes) from *Thermomyces lanuginosus* was immobilized on Lewatit VP OC 1600, a macroporous hydrophobic carrier. Three commercial lipases, namely, Novozym 435, Lipozyme RM IM and Lipozyme TL IM, and the immobilized lipase prepared in this study were evaluated for efficient synthesis of 2-EP. Our immobilized lipase was the most effective for the synthesis of 2-EP. Optimum conditions for synthesis of 2-EP were a temperature of 55 oC and enzyme loading of 2% (of the total substrate weight). The conversion of ca. 93% was achieved under these optimum conditions. There were little differences between the conversions from two temperature protocols (55 oC for 6 h; 55 oC for 1 h followed by 45 oC for 5 h). Maximum conversion of ca. 96% was obtained when molecular sieve was added in the reaction mixture.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4043) Production of Human Milk Fat Analogs According to Breast Milk Composition of Chinese Women**

Presenting Author: Sun Dongzhe - COFCO Nutrition & Health Research Institute

Triacylglycerol (TAG) is the major type of breast milk fat, which not only provides more than 50% of the energy for infants, but also has certain biological functions. Studies have shown that Chinese and Westerners differ greatly in the composition of TAGs in breast milk. Where Chinese breast milk contains higher levels of OPL than OPO. Therefore, an enzymatic transesterification method for the simultaneous production of a certain proportion of OPO and OPL was firstly established and optimized in the present study. ST58, PKOH and soybean oil were selected to synthesize the TAG skeleton of human milk fat analogs by random transesterification at first, of which the optimized substrates proportion was 75:15:10, respectively. Then, the specific transesterification at sn-1,3 positions was conducted and optimized to simultaneous produce OPO and OPL, the influences of temperature, enzyme quantity, ratio of oleic acid to linoleic acid, and the ratio of TAG to free fatty acid were evaluated. Molecular distillation was applied to purify the OPO and OPL-riched TAG

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afterwards. Reducing the dropping speed, increasing the stirring speed and temperature, as well as using secondary distillation could elevate the separation efficiency. Finally, the response surface methodology was used to calculate the maximum tolerance of palmitic acid in total fatty acids, which represented as 11.7%. Collectively, we have built and optimized the production process of human milk fat analogs according to triacylglycerol profiles of breast milk in Chinese women, and provided a reference for the industrialized production of milk powder fat suitable for Chinese infants.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3871) Establishment of a Host Vector System for the $\omega$ 3-docosapentaenoic Acid-producing Microorganism *Aurantiochytrium* Sp. T7**

Presenting Author: Brian K. H. KH Mo - Kyoto University

Omega-3 polyunsaturated fatty acids ( $\omega$ 3-PUFAs) are reported to have various physiological functions. While the physiological roles of EPA, DHA, and ALA have been well studied because of their sufficient natural supply, the biological roles of rare  $\omega$ 3-PUFAs such as SDA (18:4 $\omega$ 3), DPA (22:5 $\omega$ 3), and ETA (20:4 $\omega$ 3) are unclear due to a lack of known sources. For example, while studies have shown  $\omega$ 3-DPA to be a component of natural oils extracted from earless seal, its content was less than 5% of total lipids. Thus, recent research has focused on  $\omega$ 3-DPA production by alternative sources such as oleaginous bacteria, fungi, plants, and microalgae. We previously reported the isolation of a microorganism *Aurantiochytrium* sp. strain T7 that accumulates a significant amount of  $\omega$ 3-DPA, and optimized its culture conditions. In this study, we constructed a transformation system to elucidate the mechanism of  $\omega$ 3-DPA biosynthesis in the T7 strain. We constructed an expression vector containing a zeocin resistance gene as a selection marker and a  $\beta$ -glucuronidase (GUS) gene as an expression reporter gene. We then transformed this vector into the T7 strain by the biolistics method, and confirmed stable GUS activity in the obtained transformants. Next, we constructed another expression vector containing the fatty acid elongation enzyme gene MALCE1, and introduced it into the T7 strain by the biolistics method. MALCE1 transformants exhibited increased stearic acid accumulation, confirming the expression of MALCE1. This transformation system can now be applied for further analysis of  $\omega$ 3-DPA biosynthesis and molecular breeding of *Aurantiochytrium* sp. strain T7.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3885) *Mortierella Alpina* Molecular Breeding for Pilot-scale Eicosapentaenoic Acid Production Under Ordinary Temperature**

Presenting Author: Akinori Ando - Kyoto University

An oleaginous filamentous fungus *Mortierella alpina* 1S-4 is known to accumulate eicosapentaenoic acid (EPA, C20:5 $\omega$ 3) only when cultivated at a low temperature. However, low temperature cultivation causes slower growth. Moreover, other undesired  $\omega$ 3 fatty acids are accumulated because of the broad substrate specificity of the endogenous  $\omega$ 3 desaturase. Thus far, we isolated an EPA-producing microorganism, *Pythium torulosum*, from soil under ordinary temperature, and screened the related species based on the high ratio of EPA to arachidonic acid (C20:4 $\omega$ 6) and selected *Pythium sulcatum* and *Plectospora myriandra*. Subsequently, three putative  $\omega$ 3DS genes (Pt $\omega$ 3 from *P. torulosum*, Ps $\omega$ 3 from *P. sulcatum* and PmD17 from *P. myriandra*) were cloned from these strains. The functional analysis of these enzyme genes expressed in yeast revealed that Pt $\omega$ 3 and Ps $\omega$ 3 convert 18- and 20-carbon  $\omega$ 6 fatty acids into  $\omega$ 3 fatty acids, while PmD17 specifically convert 20-carbon  $\omega$ 6 fatty acids. Each heterologous desaturase gene modified based on codon bias of *M. alpina* were expressed in *M. alpina* and showed the same functions at 28°C. Especially, the EPA composition of transformants overexpressing PmD17 reached approximately 80% of total fatty acids at test tube scale. This transformant showed 14 g/L fatty acid productivity. The EPA production reached 52% of total fatty acids at 5 L jar fermenter scale. Furthermore, at 50 L jar fermenter pilot-scale, this transformant showed 16 g/L fatty acid productivity and EPA production reached 45% of total fatty acids. *M. alpina* could be expected as a promising EPA-producer alternative to fish oils.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4150) Tailoring Oil Palm Planting Materials for the Oleochemical Industry**

Presenting Author: Rajinder Singh - Malaysian Palm Oil Board

Palm oil and palm kernel oil are raw materials from which basic oleochemicals, namely fatty acids, fatty acid methyl esters, fatty alcohols, fatty amines and glycerol are derived. The fats and oils utilized in the oleochemical industry are those composed of fatty acids with carbon chain lengths of C8-C18. Palm kernel oil and coconut oil, which is limited in supply, are the principal representatives for C8-C14. Palm oil together with other vegetable oils (soya bean, rapeseed), and tallow oil are the main sources of C16-C18. As such, oil palm breeding has been custom-tailored to develop the appropriate planting materials which can yield higher levels of selected fatty acids. Breeding lines with kernel content up to 10%, compared to 5% observed in current commercial material, which will yield higher lauric acid are being developed via conventional breeding. High concentrate oleic acid is an important chemical requirement of the oleochemical industry. Palms with higher oleic acid content have also been identified. However, DNA markers are required to fix the traits in advanced breeding lines, in order to develop the new and

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improved varieties faster for this perennial crop. In this respect progress has been made via MPOB's genome research in identifying genomic loci linked to kernel content and the different fatty acids in selected breeding populations. The presentation will describe how these tools are being used to aid in the development of planting materials with higher content of selected fatty acids to meet the long-term requirements of the oleochemical industry.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3898) Improvement of Astaxanthin Production in *Xanthophyllomyces dendrorhous* by Resource and Metabolic Engineering**

Presenting Author: Hiroshi Kikukawa - University of Shizuoka

Red yeast *Xanthophyllomyces dendrorhous* is the only yeast known to produce astaxanthin, an anti-oxidant carotenoid widely used for the aquaculture, food, pharmaceutical and cosmetic industries. The potential of this microorganism as a platform cell factory for carotenoids production has been recognized because of high flux through its terpene pathway. Here, we studied on improvement of astaxanthin production in *X. dendrorhous* by culture engineering using citrus peel waste as a biomass resource or through development of a gene expression system in *X. dendrorhous*. As the first study, we examined the production of astaxanthin by *X. dendrorhous* using citrus peel extract as carbon and/or nitrogen sources. The yeast showed higher growth and astaxanthin production because of increase in biomass. Interestingly, *X. dendrorhous* could grow in the liquid medium containing only citrus peel extract as well as nutrient medium. It was suggested that arabinose composing polysaccharide pectin stimulated growth of *X. dendrorhous*. As the second study, we improved the astaxanthin production in *X. dendrorhous* through up-regulating the intracellular mevalonate supply by enhancing the mevalonate synthetic pathway. We developed a multiple gene expression tool using plasmids containing three strong promoters in *X. dendrorhous*. By overexpressing the five key genes of mevalonate and astaxanthin synthetic pathways using this tool, astaxanthin production was enhanced by 2.1-fold compared with the parental strain without a reduction of cell growth. Valuable resources such as citrus peel and the developed metabolic engineering tool would increase the potential of *X. dendrorhous* to become an industrial astaxanthin producer.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3912) Drug Discovery Applications of Gut Microbial Fatty Acid Metabolites**

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Presenting Author: Yasunori Yonejima - Nitto Pharmaceutical Industries, LTD.

It has been determined that gut microbes metabolically convert unsaturated fatty acids derived from dietary lipids to a variety of fatty acid derivatives. The physiological functions of these fatty acid metabolites have been gathering attentions along with an increasing interest in the relevance to the health of the host. Gut microbes catalyze the hydration, oxidation, reduction, and dehydration reactions targeting double bonds of polyunsaturated fatty acids; a broad range of fatty acids can serve as substrates, including linoleic acid, oleic acid,  $\alpha$ -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. 10-Hydroxy-cis-12-octadecenoic acid (HYA) is a gut microbial metabolite derived from linoleic acid, which is the most abundant fatty acid of vegetable oils. Miyamoto et al. reported that long-term administration of HYA has an effect of improving glucose metabolism via the fatty acid receptors GPR40 and 120 in high-fat diet load obese model mice. We now advance application studies of HYA toward industrialization and have accomplished HYA production with 50 % purity at our pilot plant. We also conducted a clinical study regarding the effect on postprandial blood glucose after the ingestion of food with HYA and confirmed the suppressive effect on the postprandial elevation of blood glucose levels. Hence, gut microbial fatty acid metabolites such as HYA are expected to have pharmaceutical applications. In this presentation, we would introduce our approach to drug discovery using gut microbial fatty acid metabolites. 1) Miyamoto J, et al., Nature communications, 2019, 10.1: 1-15 2) Yonejima Y, et al., Progress in Medicine, 2019, 39: 1147-1154

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(3915) Pennycress to Covercress: Rapid Crop Domestication with Gene Editing Technologies**

Presenting Author: Michaela G. McGinn, PhD - Covercress

Pennycress (*Thlaspi arvense*) is a common Brassica found throughout temperate regions of the world with a long list of unique attributes including an overwintering growth habit, relatively short life cycle, and naturally high oil and protein content in the up to 40 grams of seed produced per plant. CoverCress Inc. is utilizing cutting-edge gene editing technologies to rapidly domesticate and tailor this plant to serve as a cash cover crop. Elite pennycress varieties are subjected to multiple targeted genetic alterations resulting in a new variety of pennycress, Covercress, which possesses a novel lipid profile, higher seed meal quality, and earlier maturity time with no detectable mutagenesis reagent in two generations. Covercress can be planted throughout the Midwest Cornbelt within the existing corn/soybean rotation, being planted over standing corn and harvested before soybeans are planted. Using the wealth of knowledge from closely-related model plant *Arabidopsis thaliana* and what has been gleaned from standard mutagenesis and breeding experiments, new gene editing technologies can be used to quickly

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and effectively domesticate and modify high yielding pennycress germplasm into domesticated and tailor-made Covercress varieties ready to field test in under a year.

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Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Industrial Oil Products

### **(4149) Beyond FAD2: searching for additional genetic factors that contribute to cottonseed oil fatty acid composition.**

Presenting Author: Jay Shockey - SRRC, ARS, USDA

Fats, including vegetable oils, are an important component of human and animal nutritional profiles, and also serve as feedstocks in the manufacture of many industrial products. Cottonseed oil is a valuable co-product derived from the processing of cottonseed fiber. Cottonseed oils have gradually been replaced by other frying oils due to suboptimal fatty acid composition and the ban on trans-fats. Our laboratories identified a few cotton accessions with seed oils rich in oleic acid that also contain balanced ratios of saturated and polyunsaturated fatty acids, the ideal composition of a frying oil. A mutant allele of fatty acid desaturase-2 (FAD2) accounts for approximately half of the observed elevation in cottonseed oil oleic acid in these rare accessions. Currently, we are searching for additional polymorphic genes in high-oleate cotton that contribute to this useful oil trait. A number of single nucleotide polymorphisms in genes relating to lipid metabolism have been tentatively identified. Recent results in studying the links between these genes and the oil trait will be discussed.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Lipid Oxidation and Quality

### **(3496) Lipase-Assisted Production of 1-o-galloylglycerol: Characterization and its Antioxidant Properties**

Presenting Author: Siyu Zhang, MS, PhD - University of Georgia

1-o-Galloylglycerol (GG) was synthesized by lipase-assisted (Lipozyme<sup>®</sup> 435) glycerolysis of propyl gallate (PG). The reaction conditions affecting yield of GG were optimized to obtain a yield of 76.9% ± 1.2%. GG was characterized by various methods after being separated from the reaction mixture using liquid-liquid extraction. The water solubility and hydrophilicity of GG were significantly higher than those of gallic acid (GA) and PG. The antioxidant properties, measured by the ferric reducing antioxidant power (FRAP) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)

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scavenging assays, showed that GG exhibited the highest scavenging capacity (GG > GA > PG). From the results of the 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS<sup>•+</sup>) assays, GG and GA exhibited greater scavenging capacity than PG (GG = GA > PG). GG may be used as a water-soluble antioxidant in food and cosmetic applications.

Thursday, July 2, 2020

Session Time: 10:25 AM - 12:35 PM

Presentation Time: 10:55 AM - 11:20 AM

Track: Biotechnology@@@Phospholipid

### **(3955) Liquid lipase mediated production of marine based lyso-phosphatidylcholine with enriched $\omega$ -3 long chain polyunsaturated fatty acids**

Presenting Author: Zheng Guo, PhD - Aarhus University

Lyso-phospholipids enriched in n-3 polyunsaturated fatty acids have both surface/interface-active functionality and health-beneficial fragments such eicosapentaenoic acid (EPA, C20:5 $\omega$ -3) and docosahexaenoic acid (DHA, C22:6 $\omega$ -3); which could address multi-function demands in different fields. Starting with marine phospholipids, we developed an entirely green process for enzymatic production of lyso-phosphatidylcholine. A few liquid lipases are screened for ethanolysis of purified phosphatidylcholine (PC), TLC-FID and GC-FID detections were used to monitor reaction progress and examine fatty acid compositions of different product fractions. Lipase NS-40116 from a genetic *C. antarctica* lipase A (CAL-A) displayed the best performance in either yield of lyso-PC and n-3 PUFA enrichment. Critical parameters, such as ethanol:PC ratio, reaction time and temperature, water content, and enzyme dosage are optimized. The results showed that, under optimized conditions through just one step-process, the yield of lyso-PC is up to 85% and the total n-3 PUFAs enriched up to 90% and DHA up to 75% in lyso-PC fraction. <sup>31</sup>P/<sup>13</sup>C NMR analyses were used to illustrate the reaction mechanism, which verified that the regioselectivity and high non-hydrolytic property towards long chain PUFAs of CAL-A are the main character to drive this high efficiency and high selective process.

Tuesday, June 30, 2020

Session Time: 10:25 AM - 11:20 AM

Presentation Time: 10:55 AM - 11:20 AM

Track: Biotechnology@@@Processing

### **(3828) Acyl-migration Occurrence of Palm Oil During Interesterification Catalyzed by Sn-1,3 Specific Lipase**

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Presenting Author: Yong Wang, PhD - Jinan University

Interesterification is an important technical means for the modification of edible oils and fats. Fatty acids in the triglyceride molecules can be rearranged which modifies the physical properties and nutritional functions of oils and fats and hence expanding the potential application in the food industry. The use of sn-1,3 specific immobilized lipase to catalyze interesterification has the advantages of mild reaction conditions and strong specificity. However, during the reactions, change of fatty acid at the sn-2 was observed which was due to the occurrence of acyl migration. In this work, palm olein (POL) was enzymatically interesterified using Lipozyme TL IM at 50°C for 2 h and the effect of different reaction conditions (enzyme loading, reaction temperature and time) on acyl migration was investigated. The degree of acyl migration (AMD) was defined by the change in the type and content of sn-2 fatty acid. The AMD increased with the amount of enzyme loading, recording the highest AMD of 63.41% when 11% of enzyme was added. At lower temperatures (50-70°C), the AMD increased followed by reaching maximum value of 80.71% at 80°C and then decreased at 90°C. As the reaction time was increased from 0h-3h, the AMD increased and achieved 98.24% at 3h. When the reaction time was further increased, the AMD remained constant at 100%, reaching a completely randomized interesterification. It is of great significance to explore the mechanism of acyl migration during a sn-1,3 specific lipase catalyzed interesterification process as to propose a theoretical foundation for implementing a technology to monitor and control the AMD.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Processing

### **(3773) Preparation of Natural Butter Substitute by Enzymatic Interesterification from Palm-based Oils and Its Application in Whipping Cream**

Presenting Author: Wan Jun Lee - Jinan University

The base oil of a high-end whipping cream generally consists of natural butter. In order to meet the requirements of general users, the industrial application of vegetable oil-based fats continues to rise. However, vegetable oil-derived fats are significantly different in terms of their physicochemical properties compared to that of natural butter. Hence, interesterification modification is needed. Interesterified oils and fats are acquired through chemical synthesis and there are high specification requirements for both raw materials and catalysts besides of the complexity of the process. Although enzymatic interesterification (EIE) is not as frequently used in the industry, it is a far more economical and environmentally friendly method. This study aims to utilize palm oil (OL) and palm kernel oil (PKO) as raw materials and immobilized lipase Lipozyme TL IM as catalyst to produce specialty fats by EIE. Comparison in terms of the triacylglycerol (TAG) molecular species, fatty acid compositions, solid fat content (SFC), crystallization rates, crystal polymorphism, crystal microstructures and thermodynamic properties of the fat derived from natural butter to the interesterified fats were performed to explore the efficiency of EIE as a natural butter substitute. It was found that 50 %

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EIE (85 % OL+15 % PKO) + 50 % PKO had the ability to substitute natural butter due to its ideal physicochemical properties. Since immobilized lipase can be reused and the reaction conditions of packed bed reactor are mild, this study provides a theoretical basis for achieving the goal of reducing the food specialty fats production cost and meeting the consumers health needs.

Thursday, July 2, 2020

Session Time: 10:25 AM - 12:35 PM

Presentation Time: 11:45 AM - 12:10 PM

Track: Biotechnology@@@Protein and Co-Products

### **(4163) Development of germplasm lines of edible bean with improved cysteine and methionine concentration**

Presenting Author: Frédéric Marsolais, PhD - Agriculture and Agri-Food Canada, London Research and Development Centre

Objective/Hypothesis: Protein quality in common bean is limited by the suboptimal levels of sulfur-containing amino acids, methionine and cysteine. The germplasm line SMARC1N-PN1 lacks major seed storage polypeptides. This leads to increased total cysteine (up to 70%, mol per seed weight) and methionine content (about 10%) and decreased levels of the non-protein amino acid S-methylcysteine. The objective of this work was to generate germplasm lines with similar characteristics with adaptation to Manitoba. Methods used: A cross was made between SMARC1N-PN1 (S) and the navy bean cultivar Morden003 (M) to generate a population of 185 recombinant inbred lines. Protein profiles classified the lines into four groups according to genetic inheritance of phaseolin and lectin. Single nucleotide polymorphic markers enabling to track phaseolin deficiency were validated using a Kompetitive allele specific PCR assay. Lines were tested under field conditions and their amino acid concentrations were evaluated along with protein digestibility using a pH drop assay. Results: Two SS lines were recovered having a stable protein profile, 2-37 and 3-84. Line 2-37 had a 37% increase in protein digestibility corrected amino acid score as compared with parental cultivar Morden-003. Preliminary results indicated a 60% increase in protein solubility at neutral pH for the storage protein deficient line. Conclusions: the 2-37 line could be used to develop a dry bean cultivar having improved protein quality.

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Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Protein and Co-Products

### **(3864) Improving Soybean Seed Composition with Metabolic Engineering and Genome Editing**

Presenting Author: Zhan-Bin Liu, PhD - Corteva Agriscience

Soybean is a commodity crop highly valued for its protein and oil contents. Vegetable oil and protein-rich meal are two main processed products for food and feed application. Currently, soybean derives over two thirds of its value from the meal, while the oil contributes to the other one thirds of its value. Improving both protein and oil content and their compositions in soybean seeds provides an opportunity to create a next generation commodity soybean. Both metabolic engineering and gene editing technology have been used to achieve these goals. Research progress in this area will be discussed in this presentation.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Protein and Co-Products

### **(3895) A New Soybean for Quality Plant Protein**

Presenting Author: Kristin Bilyeu, PhD - Agricultural Research Service

The importance of soybean is reflected in the worldwide production of over 340 million metric tons in 2018. Soybean value depends on the position on the value chain. Soybean is processed into two main products, the vegetable oil and a protein-rich meal. While it was originally grown in the US as a forage crop, soybean became the major domestic oilseed crop up to 2005 when US nutrition facts labels were required to include trans fat content. Changes in the food industry reduced demand for commodity soybean oil. Soybean presently derives over two thirds of its value from the meal. Competition from other protein sources put pressure on soybean meal to provide increased value for livestock feed formulations. Out of this scenario is an opportunity for a new soybean that delivers increased value from functional traits for both oil and meal that deliver the highest quality plant protein. Identifying the optimum set of seed composition characteristics that improve the value of soybean while maintaining high yield potential is critical to the development of a new type of soybean. The high oleic/low linolenic acid oil trait plus the increased energy meal trait is a combination that has potential to be the next commodity soybean. Understanding the biological effects of this combination will be important to ensure more functional soybeans can be successfully deployed and utilized throughout the value chain.

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Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Protein and Co-Products

## **(3908) Protein Modification Enzyme, Protein-glutaminase as a Tool for Improving Plant Protein Usability**

Presenting Author: Shotaro Yamaguchi, PhD - Amano Enzyme Inc.

Increase of the future world population is calling for the depletion of food resources. Particularly in the protein source, in order to maintain a sustainable society, plant proteins have attracted attention than animal proteins in which energy consumption for production per unit weight is much higher than that of the plant proteins. Changes in dietary preference are also accelerating the trend. Amano Enzyme discovered protein cross-linking enzyme transglutaminase from microorganisms in the late 1980s. This enzyme has provided a new category of reaction, cross-linking, into food-grade protein modification enzymes. The main purpose is an improvement of food texture. As is well known, its practical application has had a significant impact on the world's food industry. Furthermore, we had continued to find a new protein modification enzyme from microorganism, resulting in a discovery of protein-glutaminase and successfully commercialization as a food-grade enzyme in 2000s. This enzyme, catalyzing a protein deamidation, also provided a new category into protein modification enzymes. Protein-glutaminase has been developed to improve the protein functionalities such as emulsifying and foaming properties by improving the protein solubility. Therefore, the enzyme have been expected for the modification of especially plant protein, which, in general, have lower functionality and lower usability for food. Recently, both enzymes are attracting higher expectations as a tool to promote the use of unused resource plant proteins as an alternative to animal proteins. In this presentation, development and application of protein-glutaminase will be introduced. Ref) S Yamaguchi, Biosci. Biotech. Biochem., 2017, 81(1) 54–58.

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Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Protein and Co-Products

## **(3939) Improving on perfection: multifaceted approach to improve soybean protein quality**

Presenting Author: Hari B. Krishnan - Agricultural Research Service

Soybean meal is the world's largest source of animal protein feed because of its high protein content, balanced amino acid profile, ready availability, and relatively low cost; however, its nutritional value could be further improved by enhancing the content of sulfur-containing amino acids (cysteine and methionine) and eliminating major proteinaceous anti-nutritional factors. A multifaceted approach is being employed in our laboratory to improve the nutritive value of soybean protein. These approaches include (1) the incorporation and expression of heterologous seed proteins rich in sulfur-containing amino acids, (2) genetic manipulation of enzymes involved in the sulfur assimilatory pathway, and (3) developing soybean germplasm that has drastically reduced major proteinaceous anti-nutritional factors Kunitz-trypsin inhibitors (KTI) and Bowman-Birk inhibitors (BBI). These approaches have produced encouraging results. We have generated transgenic soybean plants that show a significant increase in overall sulfur amino acid content sufficient to meet the recommended levels required for monogastric animals. Additionally, we have developed soybean experimental lines that contain a combination of two mutant genes that results in significantly lower trypsin inhibitor activity than has previously been reported. These results bode well for the development of soybean cultivars that have superior protein quality and nutritive value.

Thursday, July 2, 2020

Session Time: 8:25 AM - 10:35 AM

Presentation Time: 8:25 AM - 8:30 AM

Track: Biotechnology@@@Surfactants and Detergents

## **Introduction: Biobased Surfactants**

Co-Chair: George A. Smith, PhD - Sasol

Co-Chair: Douglas G. Hayes, PhD - University of Tennessee

Interest in biobased surfactants continues to increase yearly. This session focuses on research and development of biobased surfactants in detergents, personal care products, cosmetics, pharmaceuticals, foods, environmental remediation and other applications.

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Thursday, July 2, 2020

Session Time: 8:25 AM - 10:35 AM

Presentation Time: 8:30 AM - 8:55 AM

Track: Biotechnology@@@Surfactants and Detergents

### **(3586) Characterising Biosurfactants and Bioemulsifiers from Marine Bacteria: Structural, Functional and Biological Properties**

Presenting Author: Stephen R. Euston, PhD - Heriot-Watt University

Microbial biosurfactants and bioemulsifiers are natural surface active molecules with enormous potential as ingredients in foods and other consumer products. These molecules include low molecular weight surfactants such as the glycolipid rhamnolipids and sophorolipids and polymeric emulsifiers (polysaccharides, proteo-glycans, glyco-proteins or lipoproteins). They are said to be more functional, less toxic, more biodegradable and better for the environment than currently used, often synthetic, counterparts. If these claims are to be verified there is a need to understand better the structure-function relationships in these molecules. Their application in foods has been held back somewhat by a lack of knowledge on their functionality in multicomponent systems, and a dearth of information on their toxic effects both to humans and in the environment. We report results on the structure and properties of rhamnolipid biosurfactant and two novel polymeric bioemulsifiers obtained from marine bacterial strains. Using UHPLC-MS, HPLC-SEC and NMR we can identify the surface active rhamnolipid components and functional groups in the bioemulsifiers that suggest the latter are a glycoprotein or proteoglycan and a lipoprotein respectively. This information is linked to functional properties of relevance to foods and how rhamnolipids and microbial polymers interact with food proteins to modify emulsifying, foaming and gelation characteristics. Additionally, we report a full toxicology screen (both on human cell lines and environmental marker organisms) for the molecules. Our results give confidence that biosurfactants and bioemulsifiers are highly functional with low cyto- and environmental toxicity and show great potential as food ingredients.

Thursday, July 2, 2020

Session Time: 8:25 AM - 10:35 AM

Presentation Time: 10:10 AM - 10:35 AM

Track: Biotechnology@@@Surfactants and Detergents

### **(4208) Microbial Biosurfactants: Is Mainstream on the Horizon?**

Presenting Author: Sophie LKW Roelants, PhD - Bio Base Europe Pilot Plant

Microbial biosurfactants have been holding the promise as environmentally friendly alternatives for petrochemical derived surfactants for the last decade. Indeed, across the market (large) companies are investing in these technologies and microbial biosurfactants are already applied in quite some consumer products today. The remaining hurdles for these technologies to really lift off can be summarized as high costs in comparison to the market references and a limited variety

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in molecular structures to satisfy the plethora of sought for functionalities. Moreover, most of the technologies are still in their infancy, characterized by suboptimal processes often resulting in batch to batch variation, a lack of knowledge and a of scale up evidence. A last issue is the fact that the use of so-called first-generation renewable substrates, such as sugar and vegetable oil, as substrates negatively impacts the LCA for microbial biosurfactants. At BBEPP and InBio.be we focus on all the above-mentioned shortcomings and aim to increase the microbial biosurfactant market segment in the coming years. We apply an integrated approach where microbial strain engineering, process (fermentation and purification) development and -optimization, scale up and application testing are tightly linked and interconnected. We recently succeeded in the development of a battery of *Starmerella bombicola* yeast strains producing a library of over 20 (new-to-nature) glycolipid biosurfactants and developed sustainable and scalable (continuous) fermentation and purification processes for these biosurfactants. The biosurfactants were screened in high throughput for a range of relevant properties for the industry, such as foaming, rheology, surface tension (and CMC), emulsification, but also biological properties such as anti-microbial and -viral properties. Moreover, efforts were done to develop the bioprocesses starting from waste- and side streams instead of 1G substrates, thus positively impacting the environmental impact of the new microbial biosurfactants. The combination of the described efforts is expected to result in a commercial breakthrough of microbial biosurfactant in the next ten years.

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Surfactants and Detergents

### **(3704) Strategical production of surface-active compounds from biomass-derived carbohydrates**

Presenting Author: Vinoth Kumar Vaidyanathan - SRM Institute of Science and Technology

**Objectives/Hypothesis:** The transition towards the utilization of widely available renewable biomass for the production of useful chemicals is increasing in a way to mitigate environmental issues. In this front, by selective combination of their molecular constituents (e.g. carbohydrates) a variety of surface-active materials can be prepared. Due to their molecular constitution, these compounds are highly biodegradable and continue to evoke considerable interest as important classes of bio-based materials on account of their potential industrial applications. Contemplating these advantages, the present study utilized renewable biomass for the production of reducing sugars which were further employed in an enzymatic approach for the synthesis of surface-active compounds. **Methods:** The catalytic hydrolysis of biomass was inculcated for the release of reducing sugars, such as glucose, rhamnose, and mannose, which were then transformed into the respective glycosides by reacting with 1,3-propanediol. The esters preparation mediated by lipase was achieved by reacting the glycosides with 0.3M of fatty acid in the presence of an acyl donor. Then the surface-active compounds were extracted with chloroform and were characterized for the surface-active properties. **Results:** The produced surface-active compounds

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have the ability to reduce the surface tension of water up to 31mN/m and exhibited an emulsification index of 74%. Further, these compounds also demonstrated antimicrobial properties which could be exploited for a wide range of applications. The purity of the compounds was analyzed by HPLC and the structure was confirmed by NMR analysis. Conclusion: This work reported a promising new class of environment-friendly surface-active compounds that have various environment applications. Keywords: Surface-active compounds; Biomass; Lipase; Surface tension; Antimicrobial

Friday, January 1, 2021

Session Time: 1:00 AM - 2:00 AM

Presentation Time: 1:00 AM - 2:00 AM

Track: Biotechnology@@@Surfactants and Detergents

### **(4029) Recent developments in rhamnolipid production and application**

Presenting Author: Lu-Kwang Ju - The University of Akron

Rhamnolipids are among the best known and studied glycolipid biosurfactants with a wide range of potential applications. Fermentation productivity is a critical limitation to their development into large-scale commercial products. Addressing the productivity's high sensitivity to the limiting nutrient and its feeding control, we have recently improved the production to give a rhamnolipid concentration of 105 g/L in 144 h, corresponding to an overall volumetric productivity (including both growth and production phases) of 730 mg/(L-h). Beyond this rhamnolipid concentration, the fermentation broth became too viscous to allow suitable oxygen transfer. Repeated-cycle fed-batch fermentation, to harvest rhamnolipids at concentrations below 100 g/L to avoid oxygen transfer limitation, was further demonstrated with similarly high productivity. Specific productivity during the production phase was found to still vary between 20 and 110 mg/h per g cell dry weight. Further optimization is feasible. Besides production, we have used rhamnolipids as biopesticide in forms of aqueous solution and seed coating. The seed-coating formulation developed was demonstrated with high efficacy in controlling an important zoosporic pathogen to germination and initial growth of soybean in greenhouse. It is important to understand the rhamnolipid interactions with plants prior to its field uses in agriculture. We have therefore started to study the effects of rhamnolipid on soybeans, for example, during the imbibition and germination under different conditions. We will describe some of the key findings in our recent developments of rhamnolipid production and application.