24TH CANADIAN CONFERENCE ON FATS AND OILS

EDMONTON, ALBERTA, CANADA SEPT 26TH & 27TH, 2011

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24th Canadian Conference on Fats and Oils

TABLE OF CONTENTS

CONFERENCE PROGRAM	1
SESSION 1: EDIBLE FATS AND OILS	7
SESSION 2: NEW OILS AND OILSEED INNOVATIONS	19
SESSION 3: INDUSTRIAL LIPIDS	29
SESSION 4: PROCESSING AND SUPPLY	39
POSTER PRESENTATIONS	49

24th Canadian Conference on Fats and Oils - Program

Sunday, September 25th

6:00pm-9:00pm **Informal mixer, the Sutton Place Hotel – Winterlake Room**

Monday, September 26th

7:30-8:25 **Registration**

8:30-8:45 **Welcome address- John Kennelly**

Session I: Edible Fats and Oils

Session Chair: Roman Przybylski

8:45 **Keynote Presentation I: Brassica Oilseed Crops. Where Have We Been and Where Are We Going?** Keith Downey, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada.

9:15 **Keynote Presentation II: The Anti-Cancer Effects of the Omega-6 and Omega-3 Fatty Acids on Human Breast Cancer Cells; Implications for Novel Plant Oils.** Catherine J. Field, Julia B. Ewaschuk, Howe-Ming Yu, Marnie M. Newell. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

9:45 **New Omega-9 Sunflower Oil with Reduced Saturated Fat.** Rhonda Ryan. Dow AgroSciences Canada Inc, Calgary, AB, Canada.

10:00 **Fenugreek Seed Oil Content and Quality in Genotypes Grown in the Canadian Prairies.** James E. Thomas[1], Surya Acharya[2], Manjula Bandara[3], Crystal Snyder[4], Robin Hryniuk[4], Chris Kazala[4], Ee Lynn Lee[1,3], Rajib Prasad[1,2], and Randall Weselake[4]. [1]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada, [2]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada, [3] Crop Diversification Centre South, Alberta Agriculture and Rural Development, Brooks, Alberta, Canada, [4] Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

10:15-10:30 **Coffee break**

10:30 **The Effect of Phytosterols on the Oxidative Stability of Vegetable Oil at Frying Temperatures.** Ashutosh Singh, and Jonathan M. Curtis. Lipid Chemistry Group, Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

10:45 **Bioactive Compounds in Flaxseed.** Clara Olivia[1], Peta-Gaye Burnett[2], Denis Okinyo-Owiti[2], Mukasa Bagonluri[2], Martin Reaney[2]. [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

11:00 **Determination of Regiospecific Distribution of Fatty Acids in Milk Fat by Partial Grignard Degradation of Triacylglycerols and Gas Chromatography.** Dhananjay Zope, Paul Angers, and Joseph Arul. Department of Food Sciences and Nutrition, Université Laval, Québec, QC, Canada.

11:15 **Potential of Alkylresorcinols in Canadian Red Hard and Red Soft Wheat Bran and their Stability During Baking.** Aynur Gunenc and Farah Hosseinian. Food Science and Nutrition Division, Chemistry Department, Carleton University, Ottawa, ON, Canada.

Session II: New Oils and Oilseed Innovations

Session Chair: Randall Weselake

11:30 **Keynote Speaker III: Natural Genetic Variability for Fatty Acid Desaturases in Flax.** Sylvie Cloutier[1,2], Scott Duguid[3], Gordon Rowland[4], Mitali Banik[1], Dinushika Thambugala[2]. [1]Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada, [2] Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada, [3] Morden Research Station, Agriculture and Agri-Food Canada, Morden, MB, Canada, [4] Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada.

12:00-1:30 - Box lunch in poster area.

1:30 **Lipid Metabolism in Soybean Somatic Embryos and Plastids.** Salvatore A. Sparace, Karen R. Clark, Yan He, Tammie E. Young and Kathryn F. Kleppinger-Sparace. Department of Biological Sciences, Clemson University, Clemson, SC, USA.

1:45 **High Level Accumulation of Gamma Linolenic Acid (C18:3Δ6.9,12 *cis*) in Transgenic Safflower (*Carthamus tinctorius*) Seeds.** Cory L. Nykiforuk[1], Indra Harry[1], Olga P. Yurchenko[1], Mei Zhang[1], Catherine Reed[1], Gunamani S. Oinam[1], Steve Zaplachinski[1], Joseph G. Boothe[1], and Maurice M. Moloney[1,2]. [1]SemBioSys Genetics Inc, 110, 2985-23 Ave NE, Calgary, AB, Canada, [2]Rothamsted Research, West Common, Harpenden, Herts, United Kingdom.

2:00 **Influence of Lysophosphatidylcholine Acyl Transterase in Acyl Remodeling and Triacylglycerol Biosynthesis in Arabidopsis Seeds.** Liping Wang, Wenyun Shen, Michael Kazachkov and Jitao Zou. NRC-Plant Biotechnology Institute, Saskatoon, SK, Canada.

2:15 **Seed mutagenesis of *Brassica rapa* and *B. oleracea* for fatty acid modification in *B. napus* : Development of low linolenic acid *B. oleracea*.** Habibur Rahman. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

2:30 **Cyclolinopeptides in Flaxseed and Flaxseed Products.** Bo Gui , Mukasa Bagonluri, Martin J. T. Reaney. Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

2:45 **Bioactivity of Natural Versus Synthetic Trans Vaccenic Acid in the JCR:LA-*CP* Rat.** M. Miriam Jacome-Sosa[1], Martin J. Reaney[2], Jianheng Shen[2], Catherine J. Field[1], Donna F. Vine[1] and Spencer D. Proctor[1]. [1]Alberta Institute for Human Nutrition, University of Alberta, Edmonton, Canada, [2]College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada.

3:15 **Prepare to leave for Devonian Botanical Gardens (Bus departs at 3:15)**

4:30 - 6:25 **Reception and tours at the garden**

6:30 - 7:30 **Dinner**

7:30 - 8:00 **Presentation** – Sevim Erhan

8:00-11:00 **Musical entertainment by Jacqueline Marcelle**

Buses will leave the Devonian Botanical Garden at 9pm, 10pm, and 11pm. Drop off points include the Delta Edmonton South Hotel, the University of Alberta and the Sutton Place Hotel.

Tuesday September 27th

9:00-10:00 **Panel discussion with Sevim Erhan (USDA-ARS), Tim Haig (BIOX) and Ian Thompson (Canadian Bioenergy), moderated by Stan Blade (Alberta Innovates BioSolutions)**

10:00-10:15 Coffee break

Session III: Industrial Lipids

Session Chair: David Bressler

10:15 **Keynote Speaker III: Castor Oil: Biosynthesis and Applications.** Thomas McKeon. USDA-ARS, Western Regional Research Center, Albany, CA, USA.

10:45 **Development of Epoxides and Polyols from Renewable Resources.** Tolibjon S. Omonov, Ereddad Kharraz, Guoguang Liu, Jonathan M. Curtis. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

11:00 **Synthesis and Evaluation of a Polyol-based Biolubricant.** Jieyu Nie[1], Jianheng Shen[2], Yong Wang[3] and Martin J.T. Reaney[2]. Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [3]Department of Food Science and Engineering, Jinan University, China.

11:15 **Renewable Hydrocarbons from pyrolysis reaction of Unsaturated Fatty Acids.** Justice Asomaning, Paolo Mussone[1], Murray R. Gray[2], and David C. Bressler[1]. [1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Chemicals and Materials Engineering, University of Alberta, Edmonton, AB, Canada.

11:30 **A Novel Green Process for the Production of Biodiesel from Multi-Feedstocks.** Aijaz Baig, and Flora T. T. Ng. Department of Chemical Engineering, University of Waterloo, Waterloo, ON, Canada.

11:45 **The Development of Structural Insulating Panels using Rigid Polyurethane Foam Produced from Canola Oli.** Xiaohua Kong[1], Guoguang Liu[1], Zhijie Zhang[2], Tam Tekle[2], Jonathan M. Curtis[1]. [1]Lipid Chemistry Group, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]TTS Inc., Edmonton, AB, Canada.

12:00 **Lipid profiling of Microalgae for Biofuel Feedstock by Liquid Chromatography – High Resolution Mass Spectrometry.** Jeremy E Melanson, Karen M. MacDougall, Jesse McNichol, and Patrick J. McGinn. National Research Council of Canada, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada.

12:15-1:45 Lunch

Session IV: Processing and Supply

Session Chair: Jonathan Curtis

1:45 **Keynote Speaker V: Plant Lipid Soluble Compounds; Many Small Things Made Large through Biorefinery Processing.** Martin J. T. Reaney**.** Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

2:15 **Changes in Conjugated Linoleic Acid (CLA) and Antiradical Ability of Anhydrous Milk Fat Treated with Pressure Assisted Thermal Sterilization.** Sergio I. Martínez-Monteagudo, and Marleny D.A. Saldaña. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

2:30 **Characterization of Commercial Polyamide Membranes and their use for Lipid Separations with Supercritical Carbon Dioxide.** Oguz Akin and Feral Temelli. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

2:45-3:00 Coffee break

3:00 **Effect of the Endogenously Derived Canola Derived Antioxidants on lipid Oxidation of Canola Oil-in-Water Emulsion.** Dayanidhi Huidrom[1], Narsi Reddy[1], Ann-Dorit[1], James Friel[1], Martin Scanlon[2] and Usha Thiyam[1]. [1]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada, [2]Department of Food Sciences, University of Manitoba, Winnipeg, Canada.

3:15 **Development of a Rapid In-Situ Transesterification Method for Fatty Acid Analysis in Algae.** Julie A. Hall and Suzanne M. Budge. Food Science Program, Department of Process Engineering and Applied Sciences, Dalhousie University, Halifax, NS, Canada.

3:30 **Spectroscopic Characterization of Dimers Formed During Thermo-Oxidation of β-Sitosterol.** Ewa Sosinska, Roman Przybylski and Paul Hazendonk. Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, AB, Canada.

3:45 **Analysis of Phenolic Acids in Canola meals by ultra-Fast Liquid Chromatography-Tandem Mass Spectrometry.** Daise Lopes-Lutz, Christian Geerkens, Corinna Hofmann, Carola Tosta, Neil Brett, Robert Fowles, and Andreas Schieber. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

4:00-4:15 Closing address and graduate student awards

Session I: Edible Fats and Oils

Session Chair: Roman Przybylski

Keynote Presentation I: *BRASSICA* OILSEED CROPS. WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

Keith Downey
Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

Brassica oilseed crops have a long history of genetic manipulation by man to improve their agronomic performance and quality characteristics. Over the years the introduction of useful innovations has been accelerating. Success has largely been due to the plant's ability to accommodate genetic manipulation but also due to the development of modern analytical instrumentation and methodologies that provide ever faster and more accurate results. The importance of these factors in the identification of superior canola germplasm and varieties will be examined. A short historical review will present some of the desirable modifications achieved using older methodologies and what desirable features we may see in future varieties.

Notes:

Keynote Presentation II: THE ANTI-CANCER EFFECTS OF THE OMEGA-6 AND OMEGA-3 FATTY ACIDS ON HUMAN BREAST CANCER CELLS; IMPLICAITONS FOR NOVEL PLANT OILS

Catherine J. Field, Julia B. Ewaschuk, Howe-Ming Yu, and Marnie M. Newell
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Anti-cancer effects of n-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), on the development and progression of breast cancer have been reported. A number of metabolic intermediates, that could come from novel plant oils, exist in the n-3 and n-6 PUFA metabolic pathways, including γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), stearidonic acid (SDA) and eicosatrienoic acid (ETA). The effect of these PUFAs on breast cancer cells is unknown; likewise, the impact of pretreatment of breast cancer cells with n-3 PUFA on the efficacy of chemotherapy has not been well elucidated. Thus the objectives of our research program have been to determine the anti-cancer effects of n-3 and n-6 pathway intermediates and the impact of DHA and EPA on chemotherapy efficacy in human breast cancer cells. Several human breast cancer cell lines (MDA-MB-231, MCF-7 and SKBr-3) and the non-transformed MCF-12A breast cell line were treated with n-3 or n-6 fatty acid (α-linolenic acid, arachidonic acid, DGLA, DHA, eicosapentanoic acid, ETA, GLA, linoleic acid, SDA) for 48 hr and growth assessed. To examine cellular mechanisms, CD95 (a cell-surface death receptor) expression and localization in response to DHA and EPA (+/- chemotherapy) were assessed. In these experiments, cells were pretreated with DHA or EPA for 48 h and subjected to chemotherapy treatment for 24 h and CD95 expression and localization was assessed by western blot, flow cytometry and confocal microscopy. SDA, ETA, EPA, and DHA selectively decreased the growth of breast cancer cells by 60% ($P<0.05$), while ALA exerted no significant effect. GLA, DGLA and AA decreased the growth of cells by 60-70% ($P<0.05$), while LA did not impact growth. Membrane fatty-acid composition demonstrated that observed effects did not appear to be a result of conversion of intermediates to pathway endpoints. DHA, but not EPA, improved the efficacy of chemotherapy, and resulted in the relocation of CD95 from the cytosol to lipid rafts. Longer chain PUFAs, including pathway intermediates, appear to have an inhibitory effect on the growth of breast cancer cells, unlike their shorter precursors. DHA, but not EPA augments chemotherapy efficacy, at least in part through a CD95-mediated mechanism. Our results suggest that modifications of plant oils to provide dietary sources of n-6 and n-3 PUFA beyond ALA and LA may be of use in the prevention and treatment of breast cancer.

Notes:

NEW OMEGA-9 SUNFLOWER OIL WITH REDUCED SATURATED FAT

Rhonda L. Ryan
Dow AgroSciences Canada Inc., Calgary, AB, Canada

Consumers now realize the impact that “bad,” trans and saturated, fats have on their diet and long-term health and are looking for healthier oils. USDA 2010 Dietary Guidelines call for a reduction in saturated fat and replacing with heart-healthy monounsaturated and polyunsaturated fatty acids. Omega-9 Sunflower Oil, reduced saturated sunflower oil, was developed by Dow AgroSciences through natural breeding techniques and has less that 3.5% saturated fat and very high levels of heart-healthy monounsaturated fatty acids (Omega-9). This oil has the lowest saturated fat content of any oils on the market today and will be available for testing by food manufacturers in 2012. This presentation will review the fatty acid composition, physical properties and functionality of this new sunflower oil and compare with oils currently available. Results from a sensory study, comparing Omega-9 Sunflower Oil with 3 different commercially available oils in a stir fry application will also be presented.

Notes:

FENUGREEK SEED OIL CONTENT AND QUALITY IN GENOTYPES GROWN IN THE CANADIAN PRAIRIES

James E. Thomas[1], Surya Acharya[2], Manjula Bandara[3], Crystal Snyder[4], Robin Hryniuk[4], Chris Kazala[4], Ee Lynn Lee[1,3], Rajib Prasad[1,2], and Randall Weselake[4]
[1]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada
[2]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada
[3] Crop Diversification Centre South, Alberta Agriculture and Rural Development, Brooks, Alberta, Canada
[4] Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume which often is used as a spice and/or nutraceutical but, also is used as a source of oil to produce a maple-flavored food supplement or as an additive for some perfumes. Little information is available on seed oil characteristics for fenugreek grown in the semi-arid regions of Canada. Quality and quantity of seed oil from 10 different lines of fenugreek adapted for growth on the Canadian prairies were examined. Total lipid from 6 g samples of pre-dried fenugreek seed was analyzed by low resolution-nuclear magnetic resonance (LR-NMR). Oil contents were determined gravimetrically following lipid extraction using hexane:isopropanol. On average, seed oil content ranged from 6 to 8% (on a per weight basis); e.g., Tristar fenugreek possessed 7.5 $\pm$ 1.2 % oil (N=15) while Indian Temple possessed 6.2 $\pm$ 0.9 % (N=15), and AC Amber possessed 7.0 $\pm$ 1.0 % oil. Although large seeds generally had more oil than small seeds, this difference was small, averaging about 0.5 + 0.1%.(N=9); e.g., large and small seed from Tristar fenugreek differed in oil content by only 0.7%, while that from AC Amber differed by 0.47%. Because of the limited variation in oil contents observed, variation in seed yield (kg/ha) is likely to be the most important factor in determining oil productivity for fenugreek. Analysis of oil obtained from fenugreek indicated that the most dominant fatty acids present were 18:2 (37% - 52%), 18:3 (18% - 27%), and 18:1c9 (13% - 18%). These studies are being done in an effort to identify potential high-value markets for seed oil from fenugreek.

Notes:

THE EFFECT OF PHYTOSTEROLS ON THE OXIDATIVE STABILITY OF VEGETABLE OILS AT FRYING TEMPERATURES

Ashutosh Singh, and Jonathan M. Curtis
Lipid Chemistry Group, Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

The addition of phytosterols was found to significantly restrict oligomer formation in triolein, refined canola, high oleic sunflower and flaxseed oils heated at frying temperature for 72 hours. The antioxidative effect of phytosterols at frying temperature has been attributed to the conversion of sterols to steradiene by the 1,2 elimination of water. This theory is consistent with the observed increase in triglyceride hydrolysis, especially during the period corresponding to the appearance and growth of oligomer peaks seen by using size exclusion chromatography. Given the effectiveness of phytosterols in restricting oligomer formation at frying temperatures, ensuring presence of an optimum level of sterols in refined oils is of critical importance.

Notes:

BIOACTIVE COMPOUNDS IN FLAXSEED

Clara Olivia[1], Peta-Gaye Burnett[2], Denis Okinyo-Owiti[2], Mukasa Bagonluri[2], Martin Reaney[2].
[1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

There is substantial genetic variation in flax varieties. The Flaxseed World Core Collection (FWCC; 400 lines) was recently selected as a suitable representative subsample of the widest variation of flax gene resources. Samples of the collection grown at two locations were screened to determine the content of cyclolinopeptide (CLP) and lignan (secoisolariciresinol diglucoside - SDG). Cyclolinopeptides are cyclic hydrophobic peptides containing eight to ten amino acid residues with molecular weights of approximately 1kDa. Both CLPs and SDG have been identified as biologically active compounds. In order to determine genetic variation in both CLP and SDG fractions obtained from samples of the FWCC, the extracts were analyzed by reverse phase HPLC using UV detection at 214 nm and 280 nm. The identity of eluting peaks was confirmed by comparison of retention time and UV spectra with those of authentic CLP standards and an SDG standard. A novel CLP, found in cultivar TMP 2181 was subjected to LC-MS/MS for molecular weight and sequence identification. Cyclolinopeptides and SDG profiling data will be presented and discussed.

Notes:

DETERMINATION OF REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS IN MILK FAT BY PARTIAL GRIGNARD DEGRADATION OF TRIACYLGLYCEROLS AND GAS CHROMATOGRAPHY

Dhananjay Zope, Paul Angers, and Joseph Arul
Department of Food Sciences and Nutrition, Université Laval, Québec, QC, Canada

The stereospecific distribution of fatty acids in triacylglycerols (TAG) influence their absorption and metabolism of fats, and thus has a nutritional significance. The classical method for regiospecific analysis of milk fat, which contains short-chain fatty acids (SCFA), is pancreatic lipase deacylation. However, the use of this method for routine analysis can be laborious. Previously, we had developed a simple and fast method for regiospecific analysis of TAG in beef tallow and vegetable oils based on partial Grignard degradation of TAG, followed by dibutyrisation of the reaction products and direct analysis of the dibutyrate derivatives of monoglycerides by gas chromatography. In the present work, we applied this direct method for the determination of regiospecific distribution in the sn-1(3) and sn-2 positions of the TAG in milk fat by taking into account the response factor and minor acyl migration from secondary to primary positions on the glycerol moiety. We found that, more than 75% of the SCFAs (C6 to C10); about 60% of the medium chain fatty acids (C12 to C15); 60-70% of the long chain saturated fatty acids (C16:0 to C18:0); 76% of oleic acid; and 51% of the linoleic acid were located at the sn-1(3) position. It was also found that 63% of the major conjugated linoleic acid (CLA) isomer, rumenic acid, was located at the sn-1(3) position. The results obtained using the present method is in agreement with the literature data. It was concluded that this method could be suitable for regiospecific analysis of milk fat. However, the determination of the position of butyric acid would require alternative alkanoyl chloride other than butyryl chloride as derivatizing agent.

Notes:

POTENTIAL OF ALKYLRESORCINOLS IN CANADIAN RED HARD AND RED SOFT WHEAT BRAN AND THEIR STABILITY DURING BAKING

Aynur Gunenc and Farah Hosseinian
Food Science and Nutrition Division, Chemistry Department, Carleton University, Ottawa, ON, Canada

Alkylresorcinols (ARs) are amphiphillic phenolic lipids and mostly found in cereal brans (e.g. rye and wheat). ARs are 1,3-dihydroxy-5-alkylbenzene homologues with odd numbered alkyl side in the range of 15–25 carbon atoms. Studies showed that by increased consumption of whole grains, there is a decreased risk of chronic diseases such as obesity, type 2 diabetes and coronary heart disease. In this study, ARs composition and content of Ontario grown Red Hard Wheat Bran (RHWB) and Red Soft Wheat Bran (RSWB) were measured, using CG-MS. All samples contained saturated and unsaturated ARs, and saturated ARs were higher than unsaturated homologues. Total ARs content for RHWB was 255.76 mg/100g before bread making and after baking the amount was 194.44 mg/100g. Before baking among ARs, C-21 was the most dominant homologue, followed by C-19, C-23, C-25 and C-17. After baking, the relative proportion of homologue C23:0 increases however C25:0 and unsaturated AR are decreased. Both these homologues are present in equal amounts before and after extraction in wheat bran. This is the first report on ARs composition of wheat grown in Canada, especially ON wheat.

Notes:

Session II: New Oils and Oilseed Innovations

Session Chair: Randall Weselake

Keynote Speaker III: NATURAL GENETIC VARIABILITY FOR FATTY ACID DESATURASES IN FLAX

Sylvie Cloutier[1,2], Scott Duguid[3], Gordon Rowland[4], Mitali Banik[1], Dinushika Thambugala[2]
[1]Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada
[2] Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada
[3] Morden Research Station, Agriculture and Agri-Food Canada, Morden, MB, Canada
[4] Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada

TUFGEN (Total Utilization Flax GENomics) is a Genome Canada funded project that aims to develop genomic resources with a broad range of applications in flax improvement and novel products. Flax seeds are high in omega 3 fatty acids praised for their health benefits in human and animal. The highly unsaturated flax oil is also used in the fabrication of green products such as linoleum and paints. To produce linolenic acid, stearic acid (C18:0) is consecutively converted into oleic (C18:1), linoleic (C18:2) and finally linolenic (C18:3) acid by stearoyl acid desaturase (SAD), fatty acid desaturase 2 (FAD2) and 3 (FAD3) enzymes. While most oilseed flax varieties have 55-57% linolenic acid content, the genetic variability for this trait is high with lines having only 2-3% (solin) and others having more than 70% (high-lin). In this part of the TUFGEN project, we focus on the genetic variability for genes encoding these three enzymes. Flax is an ancient polyploid and contains at least 2 copies of each of these genes. We cloned and sequenced all six genes (2 SAD, 2 FAD2 and 2 FAD3) from 120 accessions of flax from a core collection and analyzed the sequences for natural haplotypes and enzyme isoforms to quantify the genetic variability for these genes. The material was grown in the field at two locations in 2010 and 2011 and the oil content and fatty acid composition was measured for each line. Fatty acid composition was then correlated with haplotypes and particularly isoforms to identify the functional mutations. We also cloned and characterized a novel fad3 gene (fad3c). Transcript profiling performed on four genotypes including two high-lin, one conventional and one solin line revealed that fad3 expression is highly modulated during seed development. Also, novel loss-of-function mutations were characterized in the solin line SP2047. A heterologous yeast expression experiment demonstrated that a single amino acid change in the first His-rich motif of fad3b was sufficient to impart FAD3 loss-of-function.

Notes:

LIPID METABOLISM IN SOYBEAN SOMATIC EMBRYOS AND PLASTIDS

Salvatore A. Sparace, Karen R. Clark, Yan He, Tammie E. Young, and Kathryn F. Kleppinger-Sparace
Department of Biological Sciences, Clemson University, Clemson, SC, USA

Soybean (*Glycine max* L.) somatic embryos and their plastids are being used as a model to study the developmental relationships and metabolic interactions of fatty acid and glycerolipid biosynthesis in this seed. Batch cultures of somatic embryos exhibit sigmoidal growth kinetics over the course of eight weeks. On a fresh weight basis, embryos accumulate up to 4% protein, 2.5% soluble sugars, 1.9% starch and 1.5% lipid. Plastids with the highest rates of fatty acid biosynthesis from acetate are isolated from embryos early in exponential growth. Although soybean somatic embryos and their plastids are green, the plastids appear to function like heterotrophic plastids. Light has essentially no effect on fatty acid biosynthesis, while ATP, coenzyme A and bicarbonate are all essential. The addition of $MgCl_2$ and glycerol-3-phosphate improves activity by 110 – 120%. In the absence of added ATP, plastids are able to synthesize fatty acids in the presence of ADP + Pi at rates close to half of the ATP controls. Acetate is the preferred precursor at low (≤1 mM) concentrations while pyruvate is greatly preferred at higher concentrations (up to 10 mM). Radioactivity from acetate is recovered in primarily in palmitic and oleic acids, while radioactive fatty acids and 14C-glycerol-3-phosphate are recovered in essentially all the standard chloroplast lipids, but especially in triacylglycerol and phosphatidylcholine (23% and 20%, respectively). This research was supported by projects 8233 and 1233 from the United Soybean Board.

Notes:

HIGH LEVEL ACCUMULATION OF GAMMA LINOLENIC ACID (C18:3Δ6.9,12 *cis*) IN TRANSGENIC SAFFLOWER (*Carthamus tinctorius*) SEEDS

Cory L. Nykiforuk[1], Indra Harry[1], Olga P. Yurchenko[1], Mei Zhang[1], Catherine Reed[1], Gunamani S. Oinam[1], Steve Zaplachinski[1], Joseph G. Boothe[1], and Maurice M. Moloney[1,2]
[1]SemBioSys Genetics Inc, 110, 2985-23 Ave NE, Calgary, AB, Canada
[2]Rothamsted Research, West Common, Harpenden, Herts, United Kingdom

Gamma linolenic acid (GLA; C18:3Δ6,9,12 *cis*), also known as γ-Linolenic acid, is an important essential fatty acid precursor for the synthesis of very long chain polyunsaturated fatty acids (VLCPUFAs) and important pathways involved in human health. GLA is synthesized from linoleic acid (LA; C18:2Δ9,12 *cis*) by endoplasmic reticulum associated Δ6-desaturase activity. Currently sources of GLA are limited to a small number of plant species with poor agronomic properties, and therefore an economical and abundant commercial source of GLA in an existing crop is highly desirable. To this end, the seed oil of a high LA cultivated species of safflower (*Carthamus tinctorius*) was modified by transformation with Δ6-desaturase from *Saprolegnia diclina* resulting in levels exceeding 70% (v/v) of GLA. Levels around 50% (v/v) of GLA in seed oil was achieved when Δ12-/Δ6-desaturases from *Mortierella alpina* was over-expressed in safflower cultivars with either a high LA or high oleic (OA; C18:1Δ9 *cis*) background. The differences in the overall levels of GLA suggest the accumulation of the novel fatty acid was not limited by a lack of incorporation into the triacylgylcerol backbone (>66% GLA achieved), or correlated with gene dosage (GLA levels independent of gene copy number), but rather reflected the differences in Δ6-desaturase activity from the two sources. To date, these represent the highest accumulation levels of a newly introduced fatty acid in a transgenic crop. Events from these studies have been propagated and recently received FDA approval for commercialization as Sonova™400.

Notes:

INFLUENCE OF LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE IN ACYL REMODELING AND TRIACYLGLYCEROL BIOSYNTHESIS IN ARABIDOPSIS SEEDS

Liping Wang, Wenyun Shen, Michael Kazachkov and Jitao Zou
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Phosphatidylcholine (PC) serves not only as a major component of cellular membranes, but also a fatty acyl donor for storage lipid synthesis and a substrate for fatty acid desaturation. PC synthesized through *de novo* pathways undergoes extensive fatty acyl group remodeling through a biochemical process known as Lands cycle that involves the deacylation of PC to lysophosphatidylcholine (LPC), followed by reacylation of LPC to PC. The enzymatic component responsible for the reacylation process is the acyl-CoA: lysophosphatidylcholine acyltransferase (LPCAT). Two isoforms of LPCATs, LPCAT1 and LPCAT2, which are distributed in various tissues, are present in *Arabidopsis*. The single and double *lpcat* mutants display, to different extent, decreased linoleic acid (18:2) and increased long chain eicosenoic acid (20:1) levels in seed oil. Both thin layer chromatograghy (TLC) and lipidomic analysis reveal that the trend of fatty acid composition changes in *lpcat* double ko becomes more pronounced in developing seeds. Over-expression of either LPCAT1 or LPCAT2 in seeds gave rise to seed oil phenotype contrasting to that of the mutants. Experiments of [^{14}C]acetate and [^{14}C]oleic acid labeling show that less radio-labeled PC was detected in *lpcat* ko after short labeling time, suggesting that LPCAT mediated LPC acylation is compromised in the mutant. However, steady-state PC content in *lpcat* ko developing seeds remains unaltered when compared with wild-type. The net rate of incorporation of [methyl-^{14}C]choline into PC is 1.5~1.8 folds greater in *lpcat* ko than wild-type, which is consistent with increased transcript abundance of several key genes involved in *de novo* phospholipid synthesis. Furthermore, [methyl-^{14}C]choline pulse-chase experiment shows that PC degradation occurs much faster in *lpcat* ko than in wild-type plant, leading to a greater accumulation of radioactivity, mainly in the presence of choline, in the medium fraction. Our data reveals that LPCATs regulate PC synthesis and turnover, followed by widespread influences on glycerolipid metabolic networks in seeds.

Notes:

SEED MUTAGENESIS OF *BRASSICA RAPA* AND *B. OLERACEA* FOR FATTY ACID MODIFICATION IN *B. NAPUS* : DEVELOPMENT OF LOW LINOLENIC ACID *B. OLERACEA*.

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Genetic control of a trait in the amphidiploid species *Brassica napus* (AC genome, n=19) is often more complex compared to its diploid progenitor species, *B. rapa* (A genome, n=10) and *B. oleracea* (C genome, n=9), due to involvement of genes from its two genomes. In this species, for changing a trait to the maximum level through mutagenesis, altering the genes simultaneously of the two genomes is often required. Induced mutagenesis generally causes random changes in the DNA/genes. Therefore, in a polyploid species such as *B. napus*, it would be difficult to alter all the genes for a given trait and recover a mutant line with maximum change in the trait; however, this can be relatively easier in case of the diploid species due to involvement of gene(s) from only one genome. With the availability of in vitro cell and tissue culture techniques, creation of a *B. napus* plant from its two progenitor species is not a difficult task today. Based on these hypotheses, the present research was undertaken to alter fatty acid profile in the diploid progenitor species of *B. napus* for resynthesis of a mutant *B. napus* with maximum level of change for a given fatty acid. For this, seeds of the C genome species *B. oleracea* were treated with mutagenic agent ethyl methanesulphonate to induce mutations in genes encoding enzymes involved in fatty acid biosynthesis. M_1 plants were self-pollinated for production of M_2 seeds. A total of 8,644 M_2 plants were grown of which 66% plants produced seeds. Fatty acid analysis was done on seeds (M_3) harvested from 3,557 M_2 plants from which 16 (0.45%) M_3 seed families with 5-7% linolenic acid (wild type, ca. 10% linolenic acid) were selected. Progeny testing and selection for low linolenic acid content was done in M_3 to M6 generations from which *B. oleracea* mutant lines with 1.5-2.0% linolenic acid were obtained. To our knowledge, this is the first report on the development of low linolenic acid *Brassica* C genome species.

Notes:

CYCLOLINOPEPTIDES IN FLAXSEED AND FLAXSEED PRODUCTS

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Cyclolinopeptides (CLPs), a group of naturally occurring, hydrophobic, cyclic peptides in flax, have attracted a great deal of attention due to their immunosuppressive activity. In this study, systematic methods for CLP extraction, isolation, detection and quantification were developed. The CLPs and other hydrophobic compounds were extracted from whole flaxseed using acetone as the preferred solvent. Solid phase extraction with a silica gel column followed by selective elution with organic solvents of increasing polarity enabled the isolation of a crude peptide-rich fraction. The CLPs were separated and quantified from the peptide-rich fractions using reverse phase HPLC chromatography. The impact of flax genotype or environment on CLP production was investigated in cultivars of flaxseed. The total concentration of CLPs varied from 189 µg/g (Flanders) to 303 µg/g (Somme) in the cultivars tested. Environment, cultivar and their interaction affected the observed concentration of CLPs. The CLP distribution in flax seed coat, cotyledon and oil bodies was analyzed by HPLC. CLP concentration was higher in the cotyledon than the seed coat, but the highest concentration was observed in oil bodies. CLP levels in flaxseed oil were measured during and after oil extraction and refining. The concentration of CLPs was higher in expeller-extracted crude oil and solid foots and lower in flaxseed meal. A comparison of CLP levels in flaxseed oil extracted with a small expeller and in commercially produced flaxseed oil was performed. Crude flaxseed oil produced with a small expeller had higher levels of peptides than were observed in commercial flaxseed oil available at a local retail health food store. The effect of oil refining processes, including acid degumming and alkali refining on CLP stability, was studied. Acid degumming using 1% H_3PO_4 effectively removed all CLPs. Alkali refining was also effective at removing CLPs from the oil, although it failed to remove all peptides.

Notes:

BIOACTIVITY OF NATURAL VERSUS SYNTHETIC TRANS VACCENIC ACID IN THE JCR:LA-*CP* RAT

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T-11 vaccenic acid (VA) is a naturally occurring *trans* fat (TFA) primarily in ruminant-derived food. We demonstrated that unlike industrially produced TFA, purified VA [in the synthetic free fatty acid (FFA) form] has lipid-lowering effects, modulates fat distribution and increases energy expenditure (EE) in the JCR:LA-*cp* rat. However, it is unknown if VA has similar bioactivity in natural dairy foods, in particular when associated with other fatty acids such as saturated fat (SFA). We hypothesize that ruminant derived VA ($_r$VA), will retain health benefits compared to synthetic VA ($_s$VA) and may counteract putative effects of SFA using the JCR:LA-*cp* rat. The objectives of the study were (i) to generate an *Enriched butter* that naturally contains low SFA but high $_r$VA; (ii) a *Non-enriched butter* that contains high SFA ($_{high}$SFA), as a negative control; and (iii) to contrast the bioactivity of $_r$VA versus $_s$VA by fortifying $_{high}$SFA with $_s$VA and subsequently test the lipid modulating effects. Dyslipidemic JCR:LA-*cp* rats (n=24) were assigned to a diet for 8 weeks: 1) *Enriched butter*; 2) $_{high}$SFA butter or 3) $_{high}$SFA+$_s$VA. Results showed that obese rats fed *Enriched butter* had higher food intake ($p<0.05$) but increased EE relative the $_{high}$SFA butter group ($p<0.05$). Interestingly, body weight was not different among groups ($p>0.05$). Further, obese rats fed *Enriched butter* had lower plasma TG ($p<0.05$), total cholesterol (TC) ($p<0.01$) and LDL-C ($p<0.05$) relative to the $_{high}$SFA group. Obese rats fed $_{high}$SFA+$_s$VA butter had reduced plasma TC as compared to the $_{high}$SFA butter. In conclusion, $_r$VA as a TG retains health benefits by increasing EE and improving plasma lipid profile relative to the FFA form. Moreover, regardless of SFA content, VA (ruminant or synthetic) decreased plasma TC relative to $_{high}$SFA butter. Therefore, we speculate that the ratio of rVA to SFA in dairy foods may be important for health and hypolipidemic properties.

Notes:

Session III: Industrial Lipids

Session Chair: David Bressler

Keynote Speaker IV: CASTOR OIL: BIOSYNTHESIS AND APPLICATIONS

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The castor seed produces an oil containing up to 90% ricinoleate (12-hydroxy-oleate). Castor oil is the sole commercial source of ricinoleate, and uses for castor oil and ricinoleate include greases and other lubricants, surfactants, drying oils, emollients, engineering plastics, thermoplastics and insulators. Other industrial uses for hydroxy fatty acids are limited by their availability. For example, castor oil methyl esters are excellent lubricity additives (0.5%v/v) for diesel fuel, eliminating the need to add sulfur compounds that impart lubricity. The volume of castor oil needed to provide this additive for US diesel applications is over 15 times the current level of US imported castor oil. The presence of the toxic protein ricin and highly allergenic storage proteins are significant obstacles to growing and processing castor. As a result, there is great interest in understanding how castor produces a high ricinoleate oil. Others have shown that the oleoyl hydroxylase (FAH) gene is not sufficient to produce a high ricinoleate oil in transgenic plants. We found that microsomes from castor endosperm demonstrate a preference for inserting ricinoleate into TAG, suggesting that the final step in oil biosynthesis provides some selectivity for acylating endogenous diacylglycerols. We have evaluated the expression and activity of DGAT type 1 from castor (RcDGAT1) and found that it had a slight preference for ricinoleate caylglycerols. Others have shown that RcDGAT2 shows stronger preference for ricinoleate substrates than RcDGAT1 and demonstrated increased incorporation of ricinoleate in oils of transgenic plants. Other factors involved in transgenic production of a castor oil substitute will be discussed in terms of enzymes that display the preference for producing or using ricinoleate that underlies the ability of the castor seed to make such an unusual oil.

Notes:

DEVELOPMENT OF EPOXIDES AND POLYOLS FROM RENEWABLE RESOURCES

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At present, the majority of the commercially available polymers and monomers are derived from non-renewable, petrochemical feedstocks. The development of cost effective alternatives from renewable resources is desirable and would likely find many commercial opportunities; also due to the increased concerns regarding environmental sustainability.

The overall goal of this research was the modification of vegetable oils via epoxidation of double bonds using oxidizing agent in combination with an acid. Synthesized epoxides (mainly canola oil based) were utilized as an inter-fiber adhesive bonding to make bio-composites; as an alternative way to manufacture formaldehyde-free fiber- and particle-board panels.

Synthesised epoxides from different oils were also used to produce the polyols with the diverse physical-chemical properties. Feasibility of the canola oil based epoxide to make variety of polyols via hydroxylation process has also been shown also in pilot scale. Moreover, these polyols have been applied to make different flexible and rigid foams and plastics for multiple applications, such as for structural insulations, coatings, adhesives.

Notes:

SYNTHESIS AND EVALUATION OF A POLYOL-BASED BIOLUBRICANT

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Vegetable oils are typically over 95% triacylglyceride (TAG) a potential low-cost feedstock for biolubricant production. The properties of lubricants produced from TAG are strongly influenced by the chemical properties of glycerol and the fatty acid profile. When compared with commercial lubricant oils vegetable oil has poor low temperature performance and oxidative stability. These properties limit the application of vegetable oil directly as lubricants. In this work, the glycerol backbone of vegetable oil was replaced by trimethylolpropane (TMP), a colorless triol, via a two-step reaction. Optimization of the reaction afforded 100% yield of triester in the crude product. Triesters and excess fatty acid methyl ester (FAME) present in the crude reaction mixture were separated by semi-preparative reverse phase high performance liquid chromatography (HPLC). The low-temperature performance of enriched TMP triesters was analyzed by 500 MHz ^{1}H-NMR. Also the oxidative stability index of the crude mixture was determined. The results suggest that biolubricant with improved low-temperature performance and good oxidative stability may be produced from vegetable oils by synthesis of fatty acid polyesters on alternative polyols.

Notes:

RENEWABLE HYDROCARBONS FROM PYROLYSIS REACTION OF UNSATURATED FATTY ACIDS

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The aim of this study was to identify the dominant chemical pathways for the pyrolytic conversion of two unsaturated fatty acids, oleic and linoleic acid, occurring in animal and vegetable triglycerides. The gas, liquid and solid products of batch reactions conducted in micro-reactors over a range of temperatures and reaction times were extracted and analyzed with a combination of gas chromatography and mass spectrometry techniques. This presentation will discuss in detail the effect of reaction temperature and residence time on the product stream distribution and will propose a reaction scheme to account for a complex network of sequential and concurrent reactions such as of decarboxylation, decarbonylation, cracking, hydrogenation, and isomerization reactions. A total mass balance based on quantitative chromatography data analysis of the pyrolysis products will also be presented. The work outlined in this presentation consolidates and expands previous findings from our laboratory and demonstrates the industrial potential and process flexibility of fatty acids pyrolysis for the production of renewable hydrocarbons.

Notes:

A NOVEL GREEN PROCESS FOR THE PRODUCTION OF BIODIESEL FROM MULTI-FEEDSTOCKS

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Biodiesel is a green, nontoxic, renewable, and biodegradable alternative green fuel for petroleum-based diesel. However, the major obstacles for the production of biodiesel at an industrial scale are the high production cost, expensive feedstocks, limited local availability, and complex production processes. The high production cost of biodiesel is related to the relative high price of the refined vegetable oils used. This problem can be addressed by using low-cost feedstocks, such as waste oils and fats. However, these feedstocks contain a high amount of free fatty acids (FFA), which cannot be used for the production of biodiesel using a traditional homogeneous alkali-catalyzed transesterification process. Furthermore, the production cost of biodiesel is also becoming more significant, particularly, when a feedstock with high FFA content is used. Hence, there is a need for new innovative technologies with reduced processing cost. We have developed a novel green technology for the production of biodiesel using a simple and environmentally green single-step solid acid-catalyzed process to produce high quality biodiesel from multi-feedstocks including yellow grease. It was found that the FFA present in the yellow grease were converted to biodiesel with a 95% conversion using a solid acid catalyst. Furthermore, the yellow grease was successfully transesterified to produce a product with 87.3 mass% ester content. Analysis based on the ASTM D974, and EN14103 standards confirmed the production of high-purity biodiesel from yellow grease with only 3% linolenic ester which is far below the limit of EN14103. The recycling studies shows even after 5 reaction cycles, catalyst activity remains at 97% of the fresh catalyst. This demonstrates the reusability of this novel catalyst. Also, major process parameters were investigated including FFA content in the feedstock. This green technology has potential for industrial-scale production of biodiesel from multi-feedstocks.

Notes:

THE DEVELOPMENT OF STRUCTURAL INSULATING PANELS USING RIGID POLYURETHANE FOAM PRODUCED FROM CANOLA OIL

Xiaohua Kong[1], Guoguang Liu[1], Zhijie Zhang[2], Tam Tekle[2], and Jonathan M. Curtis[1]
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Structural insulating panels (SIPs) are composite construction materials consisting of an insulating layer of rigid polymer foam sandwiched between two layers of structural board. Polyurethane (PU) foam is the best insulating material amongst all of the rigid foams commonly used for SIPs. Conventional PU foam is produced by the reaction between polyols and isocyanates, with other additives such as blowing agents, catalyst and surfactant added to modify reaction conditions and produce a foam with desirable properties. With the increasing concern over the depletion of non-renewable resources, it would be desirable to replace petroleum-derived ingredients in materials like PU foam formulations. In this work, novel polyol mixtures (Liprol ™) were produced from epoxidised canola oil via an inexpensive process. Liprol, which is derived from a renewable resource, was used to replace 100% of the petroleum derived polyols in the foam panel preparation. The optimum Liprol composition was selected by exploring the differences between the reactions that form PU foams from commercial polyol formulations to those using the canola-derived Liprol. The properties of PU insulating foams in terms of compressive strength, R-value, closed-cell content, thermal mechanical properties and ease of production were evaluated. The overall properties of PU insulation foams prepared from canola-derived Liprol were found to be comparable with those of commercial PU foams.

Notes:

LIPID PROFILING OF MICROALGAE FOR BIOFUEL FEEDSTOCK BY LIQUID CHROMATOGRAPHY – HIGH RESOLUTION MASS SPECTROMETRY

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Renewable fuels from algae have recently been gaining interest as a viable carbon-neutral alternative to fossil fuels. Typically, characterization of algal feedstock involves breaking down intact lipids, followed by derivatization of the resulting fatty acids to fatty acid methyl esters prior to analysis by gas chromatography (GC). However, knowledge of the intact lipid profile could offer significant advantages for discovery stage biofuels research such as the selection of an algal strain or the optimization of growth and extraction conditions. For instance, strains that yield abundant triacylglycerols relative to phospholipids are generally preferable to biofuel production.

In this presentation, a comprehensive workflow for screening microalgal strains suitable for biofuel production will be described. High performance liquid chromatography coupled to charged aerosol detection (HPLC-CAD) was employed as an initial screen to determine lipid classes. Strains yielding high levels of TAG were subjected to further characterization by HPLC combined with high-resolution mass spectrometry. Complete profiles for several lipid classes were analyzed in the same chromatographic run, using a combination of accurate mass and diagnostic fragment ions for identification. Using this approach, greater than 100 unique TAGs were identified over the range of algal strains studied and TAG profiles were obtained to assess their potential for biofuel applications. Under the growth conditions employed, *Botryococcus braunii* and *Scenedesmus obliquus* yielded the most comprehensive TAG profile with a high-abundance of TAGs containing oleic acid, which is favourable for biodiesel production. The methodology was then employed to measure changes in specific TAG concentrations in response to varying algal culturing conditions.

Notes:

Session IV: Processing and Supply

Session Chair: Jonathan Curtis

Keynote Speaker V: PLANT LIPID SOLUBLE COMPOUNDS; MANY SMALL THINGS MADE LARGE THROUGH BIOREFINERY PROCESSING

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Biorefinery processes are designed for thorough fractionation of biological materials for total utilization. New biorefinery processes that were developed to process existing crops are generating fractions that have unique chemistry and potentially unique applications. Currently, we are exploring the polar lipids present in fractions produced by biorefinery processing of oilseeds grown in Western Canada. Lipid soluble compounds, their sources and many potential applications will be discussed.

Notes:

CHANGES IN CONJUGATED LINOLEIC ACID (CLA) AND ANTIRADICAL ABILITY OF ANHYDROUS MILK FAT TREATED WITH PRESSURE ASSISTED THERMAL STERILIZATION

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Anhydrous milk fat (AMF), a versatile dairy ingredient with various applications in confectionary, bakery and dairy products, is the major dietary source of conjugated linoleic acid (CLA) in human diet. CLA are positional and geometrical isomers of linoleic acid having a conjugated double-bond system. Epidemiological studies have positively related CLA intake with health-promoting and disease-preventing properties. The concentration of CLA in AMF can be markedly enhanced through diet manipulation and nutritional management of dairy cow. Unfortunately, CLA is not stable upon thermal processing and significant losses in terms of biological activity occurred through oxidation and isomerization. Pressure assisted thermal sterilization (PATS) of preheated sample (~60-110°C) reduces the severity of thermal treatments, preserving the biological activity of functional compounds. In this study, the effects of pressure (100, 350 and 600 MPa), temperature (60, 90 and 120˚C) and treatment time (3.5, 8.5 and 13.5 min) on conjugated linoleic acid (CLA) content and antiradical ability were evaluated in anhydrous milk fat (AMF) rich in CLA. At 100 MPa, CLA was relatively stable over 13.5 min regardless the temperature (80-95% of retention). Similarly, relative high retention values of CLA (85-95%) were obtained after 13.5 min at 60°C in the pressure range of 350 to 600 MPa. Contrary, only 40.25 ± 2.1% of CLA was retained after 13.5 min at 600 MPa and 120°C. Interestingly, CLA preserves its antiradical ability measured by DPPH technique after 14 min of treatment at 600 MPa and 120°C. Thus, after a pressure treatment, CLA can still donate hydrogen to form a CLA free radical that further reacts to inhibit hydroperoxides formation. For the first time, the effect of pressure assisted thermal sterilization conditions on CLA retention of enriched AMF was studied.

Notes:

CHARACTERIZATION OF COMMERCIAL POLYAMIDE MEMBRANES AND THEIR USE FOR LIPID SEPARATIONS WITH SUPERCRITICAL CARBON DIOXIDE

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Integrating a membrane separation unit to supercritical extraction process has great potential for energy savings throughout processing. Although polymer membranes offer great advantage for SC-CO_2 recycling, thus far, there is no polymer membrane available specifically produced for separation of lipids during SC-CO_2 processing.

The objectives of this study were to characterize two commercially available RO membranes, AK and SG, using contact angle, FE-SEM, XPS and ATR-FTIR measurements, and to investigate their performance by measuring triacylglycerol (TAG) and oleic acid (OA) retention, CO_2 flux, TAG/OA separation factor and total lipid yield under specified conditions.

AK membrane exhibited the characteristic properties of polyamide membranes formed via interfacial polymerization of m-PDA and TMC monomers. For the SG membrane, piperazine and m-PDA blend was most likely used as monomers, which underwent esterification with PVA during thin film formation. High contact angle of AK was due to extensive intermolecular hydrogen bonding whereas SG membrane was lacking most of the amide hydrogen and carboxyl groups due to the different protocols employed during its fabrication.

TAG and OA retention and CO_2 flux were determined at 120 and 280 bar. Higher pressure processing caused a higher decrease in flux in a shorter processing time for both membranes at constant transmembrane pressure. Increase in flux with increasing transmembrane pressure was higher at lower feed pressures. Flux of AK membrane decreased much faster than that of SG membrane independent of pressure conditions during processing with OA. Pure SC-CO_2 was fed to the system at certain time intervals to decrease the cake formation, which was effective for obtaining higher flux during separations at only lower feed pressures.

Notes:

EFFECT OF THE ENDOGENOUSLY DERIVED CANOLA DERIVED ANTIOXIDANTS ON LIPID OXIDATION OF CANOLA OIL-IN-WATER EMULSION

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Lipid oxidation is one of the detrimental factors in affecting the quality of food. Various attempts have been made to retard lipid oxidation; physical as well as chemical means. One of the most effective ways is the use of antioxidants. Most active antioxidants used in industries are mostly synthetic and, thus, raise its health concern. Therefore, natural and healthy antioxidants that effectively control oxidation are now on search. Lipid in food mostly exists in the forms of emulsion. Emulsion is formed when two (or more) immiscible liquids are dispersed over each other. Emulsions are thermodynamically unstable mixture due to high surface tension. Surface active agents called emulsifiers are generally used to reduce surface tension to keep it kinetically stable for certain amount of time. The present project aims at evaluating the effect of canola (*Brassica juncea*) derived antioxidants on whey protein stabilized canola oil in water emulsion at elevated temperature. 10 % canola oil in water emulsion using 1% whey protein as emulsifier was first prepared by passing through homegeniser. Antioxidants (sinapic acid extract. SA(E) and whole extract, WE) at two concentrations (100 and 350 µM) were added and incubated at 30°C. Sinapic acid and Butylated hydroxyl toluene (BHT) standards were also used as reference. Oxidation markers like peroxide value and conjugated dienoic % are monitored to assess the anti-oxidative effect. BHT was found to be the most effective antioxidants. The effectiveness of antioxidants in terms of peroxide value measurement are in the order: BHT-350>WE-350>WE-100>BHT-100. The same order is followed in conjugated dienoic acid measurement. This also indicates that WE has potential to replace other synthetic AO though not as powerful as them. The physical stability of the emulsion was determined by particle size measurement. The particle size of oil droplets remains constant throughout experimental period. Further research will focus on the effect of other anti-oxidative bioactives like sinapine and canolol.

Notes:

DEVELOPMENT OF A RAPID IN-SITU TRANSESTERIFICATION METHOD FOR FATTY ACID ANALYSIS IN ALGAE

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The increased focus on algal oil as a source of omega-3 fatty acids has created a need for a rapid testing method for algae samples, as current methodologies are time-consuming and may promote sample loss. Traditional algal lipid profiling requires two individual processes: lipid extraction with large volumes of organic solvents, followed by conversion of lipid into fatty acid methyl esters (FAME) to be analyzed by gas chromatography. In this research, a rapid *in-situ* method of transesterification is studied, where algal lipids are converted to FAME within one reaction vessel without extracting the lipids from the sample. The rapid method first transesterifies the algal lipids with 0.5 N NaOH in methanol, followed by 14% BCl_3 in methanol reacted at 90 °C for 10 min to form FAME and to neutralize the base catalyst to prevent saponification. To ensure the proficiency of the rapid method, algal FAME was prepared using both the rapid method and the traditional method of Folch et al. lipid extraction followed by sulphuric acid-catalyzed transesterification. The rapid method demonstrated a 13% higher conversion of lipids compared to the traditional method. To improve the efficacy of the rapid method, four variables were independently investigated to find the optimal range of operating conditions: water content of the sample, sample load limit, reaction time, and reaction temperature. Using samples of algal culture and extracted algal lipid, water contents up to 0.5 mL were not found to limit the reaction, while the limit of lipid load was found to be approximately 1 mg. The reaction time needed for the acid-catalyzed step was 10 min and its necessary reaction temperature was 90 °C, while the base-catalyzed reaction happened immediately and at room temperature. The application of this rapid method was used to select several promising algae species for omega-3 production and to compare their FA profile variations when they were grown under various temperature conditions.

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SPECTROSCOPIC CHARACTERIZATION OF DIMERS FORMED DURING THERMO-OXIADATION OF β-SITOSTEROL

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Thermo-oxidation of sterols, at temperatures typical for frying, leads to formation of oxidized derivatives, fragmented sterols, as well as oligomers. Recent research on sterol oxidation focuses mainly on the identification of oxysterol derivatives excluding compounds with high molecular mass. The aim of this work was to decipher the structure of dimers formed during β-sitosterol oxidation at 180°C in the presence of oxygen. Dimer fraction was separated by size-exclusion chromatography (SEC) after chromatographic pre-cleaning using silica gel. Three methods were used to characterize dimers configuration: 1D and 2D NMR in solution state, IR and Raman spectroscopy. NMR and IR spectra revealed loss of hydroxyl group at C-3 on the A ring with double bond at C-5 preserved. NMR data indicated that dimers are formed through ether linkage (δ_H=3.3 ppm, δ_C=76.3 ppm), confirmed by IR (1093 cm^{-1}). Furthermore HSQC and 2D COSY NMR experiments showed that ether bond is formed between C-3 and C-3' carbons of two sterol molecules. In conclusion, the predominant dimer formed during β-sitosterol oxidative degradation has configuration of 3,3'-sitosterol ether. In order to further verify chemical structure of dimers formed, analysis including application of solid state NMR and LC/MS/MS are underway.

Notes:

ANALYSIS OF PHENOLIC ACIDS IN CANOLA MEALS BY ULTRA-FAST LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Canola seeds are produced from *Brassica napus* and *Brassica rapa* cultivars, and their production in Canada is estimated to be 15 million tonnes by 2015. Canola is grown principally for its oil, which has both food and industrial applications. The meal left after oil extraction is an important high-protein feedstuff. Canola meal also contains large amounts of phenolic compounds which are known to have high antioxidant and antimicrobial activity and could have applications as natural food additives. Interactions between phenolic compounds and proteins in canola meal can cause a dark colour and undesirable taste and can also lower the nutritional value of canola meal protein, reducing the possible breadth of application for canola protein. The objective of this study was to develop a fast method of determining the profile and the amount of phenolic acids in canola meals. Five samples of canola meal from different processing facilities were analyzed. Initially, two procedures were compared for defatting of the meal using a Soxhlet apparatus and orbital shaker respectively. The orbital shaker method showed complete recovery of the oil in 1 hour at room temperature, as opposed to 8 hours of Soxhlet extraction. 70% aqueous acetone yielded the highest content of total phenolics. The optimized extraction procedure consisted of 1 g of defatted meal mixed in 30 mL of 70% aqueous acetone in an orbital shaker at 300 rpm for 3 h. After removal of acetone under vacuum, the resulting aqueous extract was submitted to solid phase extraction using C_{18} Sep-Pak cartridge. The polyphenols were desorbed with methanol, evaporated under vacuum, dissolved in methanol, and filtered into HPLC vials through nylon filters. The phenolic acids were characterized and quantified by UFLC-DAD-ESI-MS/MS as *p*-hydroxybenzoic acid (27.36-100.45 mg/100 g defatted meal), sinapine (680.70-1098.93 mg/100 g), sinapoyl glucoside (50.54-133.64 mg/100 g), sinapic acid (37.50-77.48 mg/100 g) and various sinapic acid derivatives. All phenolic acids were separated in 13 min using a Kinetex C18 column (100 x 3 mm, 2.6 µm particle size). The Folin-Ciocalteu method was also used for determination of total phenolic acids and results were expressed as sinapic acid equivalents (SAE). The content of total phenolic acids varied between 1748 and 1899 mg SAE/ 100 g.

Notes:

Poster Presentations

Edible Fats and Oils

P-1 **Effects of N-3 and N-6 Fatty Acid pathway intermediates on the Viability and Phospholipid Membrane Composition of Human Breast Cancer Cell Lines.** Howe-Ming Yu, Julia B. Ewaschuk, Marnie Newell, Vera Mazurak, Randall J. Weselake and Catherine J. Field, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-2 **Binding Interaction of Cyclolinopeptides to Human Serum Albumin.** Youn Young Shim, Denis P. Okinyo-Owiti, Jianheng Shen, Pramodkumar D. Jadhav, and Martin J.T. Reaney, Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

P-3 **Association of Lipid Oxidation Assays with Tocopherol isomer Activity in Different Oils.** Ingrid Elisia[1], John W. Young[1], and David D. Kitts[1], [1]Department of Food Nutrition and Health, University of British Columbia, Vancouver, B.C, Canada.

P-4 **Effects of Milk Ultrafiltration on the Quality of Dutch Cheese with Reduced Fat Content During Ripening.** Michael Olkowski[1,2], Antoni Pluta[1], Anna Berthold-Pluta[1], Martin J.T. Reaney[2], [1]Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Warsaw, MAZ, Poland, [2]Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.

P-5 **Choline Deficiency Impairs Intestinal Lipid Metabolism in Lactating Rat.** Nusaibah Ab Aziz, Karen B. Kelly, Mélanie Légaré, Kelly-Ann Leonard, Neele Dellschaft, Sue Goruk, Jonathon Curtis, Donna Vine, Spencer Proctor, Catherine Field, René L. Jacobs, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-6 **Flax Cyclolinopeptides are Free Radical Scavengers.** Oyunchimeg Sharav*[1],Peta-Gaye G. Burnett[2], Denis P. Okinyo-Owiti[2], Ramaswami Sammynaiken[3], Martin J. T. Reaney[2], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [3]Department of Chemistry, SSSC, University of Saskatchewan, Saskatoon, SK, Canada.

P-7 **Ruminant Trans Fat Trans-11 Vaccenic Acid is More Bioavailable from a Triacylglycerol Source and Acts as a Natural peroxisome Proliferator-Activated Receptor Ligand in a Rodent Model of Dyslipidenia and metabolic Syndrome.** Ye Wang[1,2], M.Miriam Jacome-Sosa[1,2], Martin J. Reaney[3], Jianheng Shen[3], Shannon Scott[4], Catherine J. Field[2], Donna F. Vine[1,2] and S.D Proctor[1,2], [1]Metabolic and Cardiovascular Diseases Laboratory, University of Alberta, Edmonton, AB, Canada, [2]Alberta Institute for Human Nutrition, University of Alberta, Edmonton, AB, Canada, [3]Department of Plant Science, University of Saskatchewan, Saskatoon, SK, Canada, [4]Brandon Research Centre, Agriculture and Agri-Food Canada, Brandon, MB, Canada.

P-8 **Validation of a LC-MS/MS Method for the Quantification of Choline-Related Compounds and Phospholipids in Foods and Tissues.** Yeping Xiong, Yuan-Yuan Zhao, Sue Goruk, Kirsten Oilund, Catherine Field, Rene Jacobs, and Jonathan Curtis, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-9 **Docosahexanoic Acid Alters Cell Death, Cancer and Cell-Cycle Signaling Networks in Breast Cancer Cell Lines.** Julia B. Ewaschuk, Rene Jacobs, Randy Nelson, Marnie Newell, and Catherine J. Field, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-10 **Obtaining Milk Fat Fractions Enriched in Conjugated Linoleic Acid.** Sergio I. Martínez-Monteagudo, Mohamed Khan, Marleny D.A. Saldaña* and Feral Temelli, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

New Oils and Oilseed Innovations

P-11 **Simultaneous Over-Expression of FATB and Silencing of SADs by Artificial miRNAs Increases Saturated Fatty Acid Levels in *Brassica napus Seeds.*** Jin-Yue Sun, and Mark A. Smith, Plant Biotechnology Institute, National Research Council Canada, Saskatoon, SK, Canada.

P-12 **Expression of a Novel *Arabidopsis thaliana* Phospholipase A_2 cDNA in Yeast.** Guanqun (Gavin) Chen[1], Ida Lager[2], Jenny Lindberg Yilmaz[3], Crystal L. Snyder[1], Anders S. Carlsson[2], Sten Stymne[2], Randall J. Weselake[1], [1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden, [3]Scandinavian Biotechnology Research (ScanBiRes) AB, Alnarp, Sweden.

P-13 **Isolation, Extraction and Purification of Cyclolinopeptides D", F" and G".** Vijaya P. Jadhav, Peta-Gaye G. Burnett, Jianheng Shen, Denis P. Okinyo-Owiti, Martin J.T. Reaney, Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

P-14 **Flax Cyclic Peptides with Fluorescent Tags.** Pramodkumar D. Jadhav[1], Ramaswami Sammynaiken[2], Denis P. Okinyo-Owiti[3], Jianheng Shen[3], Peta-Gaye Burnett[3] and Martin J.T. Reaney[3], [1]Department of Food and bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Saskatchewan Structural Science Centre, University of Saskatchewan, Saskatoon, SK, Canada, [3]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

P-15 **Comparative Genome Analysis Reveals B3 Transcription Factors are Prominent in Brassicaceae and major Crop Species.** Fred Y. Peng, Randall J. Weselake, Agricultural Lipid Biotechnology Program, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-16 **Identification of Genes Involved in Lipid Biosynthesis in Developing Seeds of Sea Buckthorn (*Hippophae rhamnoides L.*).** Crystal L. Snyder[1], Tahira Fatima[2], Randall J. Weselake[1] and Priti Krishna[2], [1]Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Department of Biology, University of Western Ontario, London, ON, Canada.

P-17 **Putative Regulatory Role of the N-Terminal Domain of *Brassica napus* Diacylglycerol Acyltransferase 1 (DGAT1).** Nidhi Sharma, Michael S. Greer, Martin Truksa, Wei Deng and Randall J. Weselake, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-18 **The Effect of Oxygen and Light on Oil Production in Developing *Brassica napus* Seeds.** Sherry L. Lawson, Nidhi Sharma, and Randall J. Weselake, Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-19 **First Report of Outcrossing in Camelina (*Camelina sativa L.* Crantz), a potential platform for Bioindustrial Oils.** Kimberly D. Walsh[1], Debra M. Puttick[2], Melissa J. Hills, Rong-Cai Yang[1], Keith C. Topinka[1], and Linda M. Hall[1], [1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Linnaeus Plant Sciences Inc; and [3]Department of Biological Sciences, Grant MacEwan University, Edmonton, AB, Canada.

P-20 **Recombinant Expression of Plant Diacylglycerol Acyltransferases from Sea Buckthorn Pulp and Cocoa Bean.** Ying Zhang[1], Rodrigo M. Siloto[1], Tahira Fatima[2], Priti Krishna[2] and Randall J. Weselake[1], [1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Department of Biology, University of Western Ontario, London, ON, Canada.

P-21 **Transcript Profiling of Genes Involved in Triacylglycerol Assembly in Flax (*Linum usitatissimum* L.).** Aruna D Wickramarathna, Rodrigo M Siloto, and Randall J Weselake, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-22 **Increasing Seed Oil Content through Enzyme Engineering.** Rodrigo M. P. Siloto and Randall J. Weselake, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-23 **Bioactive Oils Program – Adding Value to Canada's Oilseeds.** E. Chris Kazala[1], Linda M. Hall[1], Roman Przybylski[2], Xiao Qiu[3], Habibur Rahman[1], Andreas Schieber[1], Saleh Shah[4], Stuart Smyth[5], Mark Smith[6], Feral Temelli[1] and Randall J. Weselake[1], [1]Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, AB, Canada, [3]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [4]Alberta Innovates Technology Futures, Vegreville, AB, Canada, [5]Department of Bioresource Policy, Business & Economics, University of Saskatchewan, Saskatoon, SK, Canada, [6]National Research Council – Plant Biotechnology Institute, Saskatoon, SK, Canada.

P-24 **Bioactive Novel Cyclinopeptides in Flax seed** Peta-Gaye G. Burnett[1], Denis P. Okinyo-Owiti[1], Lester W. Young[1], Sandra Stone[2], Raju Datla[2], Michael K. Deyholos[3], and Martin J.T. Reaney[1] [1] Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada. [2] National Research Council Plant Biotechology Institute, Saskatoon, SK, Canada. [3] Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada.

Industrial Lipids

P-25 **Fermentation of Stillage.** Kornsulee Ratanapariyanuch[1], Youn-Young Shim[2], Monique Haakensen[3], Martin J.T. Reaney[2], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [3]Contango Strategies Ltd., Saskatoon, SK, Canada.

P-26 **Development of Sodium Alkoxide Catalysts from Polyols for Biodiesel Production.** Felicia H. Y. Gok[1], Gabrielle Schatte[2] and Martin J.T. Reaney[3], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, [2]Saskatchewan Structural Science Centre, University of Saskatchewan, 110 Science Place, Saskatoon, SK, Canada, [3]Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada.

P-27 **Production of Biolubricant (Trimethylolpropane Esters) from Fish Stearin.** Deyun Yuan[1], William W. Riley[1], Yong Wang[1], Jianheng Shen[2], Jieyu Nie[3], and Martin J.T. Reaney[2], [1]Department of Food Science and Engineering, Jinan University, Guangzhou, China, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [3]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

P-28 **An Investigation into the Ozonolysis of Simple Lipids by LC/MS and GC/MS.** Chenxing Sun, Yuanyuan Zhao, and Jonathan M. Curtis, Lipid Chemistry Group, Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-29 **The use of Non-Aqueous Reversed Phased LC/MS to Monitor the Formation of Intermediates and the Reaction Kinetics During the Epoxidation of Canola Oil.** Tuan Nurul Sabiqah Tuan Anuar[1], Yuan-Yuan Zhao[1], Guoguong Liu[1], Jonathan M. Curtis[1], [1]Lipid Chemistry Group, Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-30 **Characterization of Byproducts Formed from Pilot Scale Canola Oil Epoxidation by LC/MS/MS.** Yuan-Yuan Zhao, Guoguang Liu, Ereddad Kharraz, Tolibjon Omonov, Paul Tiege, and Jonathan M. Curtis, Lipid Chemistry Group, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-31 **Biodiesel Process Development for Industrial Applications: Alternative Feedstocks, Emerging Green technologies, and Quality Challenges.** Aijaz Baig, and Flora T. T. Ng, Department of Chemical Engineering, University of Waterloo, Waterloo, ON, Canada.

P-32 **A Simple and Green Analytical Method for the Determination of Acid Number of Biodiesel and Biodiesel Blends using Green Chemistry Approaches.** Aijaz Baig[1], Mike D. Paszti[2], and Flora T. T. Ng[1], [1]Department of Chemical Engineering, University of Waterloo, Waterloo, ON, Canada, [2] Rothsay, a Division of Maple Leaf Foods Inc., Guelph, ON, Canada.

P-33 **A direct Method for the Synthesis of Fatty Acid Methyl Ester (FAME) from Crude Jatropha Oil as Second Generation Feedstock using Versatile Green Catalysts.** Aijaz Baig, and Flora T. T. Ng, Department of Chemical Engineering, University of Waterloo, Waterloo, ON, Canada.

P-34 **Enzymatic Conversion of Corn Oil to Biodiesel in Supercritical Carbon Dioxide.** Ozan Nazim Ciftci and Feral Temelli, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-35 **The Effect of Growth Medium pH on Oil Accumulation in Several Isolates of *Chlorella* Native to the Canadian Praries and the Athabasca Oil Sands.** Blair Skrupski[1], Kenneth Wilson[2], and Jitao Zou[1], [1]National Research Council Plant Biotechnology Institute, Saskatoon, SK, Canada, [2]Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada.

Processing and Supply

P-36 **Development of a Continuous Crystallization Process for the Concentration of Omega-3 Fatty Acids from Mackerel Oil.** Prerna Sinha[1], Paul Angers[2], and Joseph Arul[2], [1]Department of Chemical Engineering, Université Laval, Québec, Qc, Canada, [2]Department of Food Science and Nutrition, Université Laval, Québec, Qc, Canada.

P-37 **$SCCO_2$ and Lignans from Flaxseed: Extraction Yield and Effect of Seed Pretreatment.** Lauren Comin, Feral Temelli and Marleny Saldaña, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-38 **Minimal Refining of Canola Oil: Effects on Nutrients.** Saeed Mirzaee Ghazani and Alejandro G Marangoni, Department of Food Science, University of Guelph, Guelph, ON, Canada.

P-39 **Optimization of Enzymatic Hydrolysis of Sacha Inchi Oil.** Glaucia H. C. Prado and Marleny D. A. Saldaña, Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-40 **High Throughput HPLC Analysis of Cyclolinopeptides in Flax Seed.** Mukasa T. Bagonluri[1], Denis P. Okinyo-Owiti[1], Peta-Gaye G. Burnett[1], Clara Olivia[2], Bo Gui[2], and Martin J.T. Reaney[1], [1]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

P-41 **Novel Polysaccharide Aerogels using Supercritical Carbon Dioxide: Application as Flaxseed Bioactive Carriers.** Lauren Comin, Feral Temelli and Marleny Saldaña, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

P-42 **Physical Properties of Carbon Dioxide-Expanded Canola Oil and its Blend with Canola Stearin.** Ehsan Jenab and Feral Temelli, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

Poster Presentation Abstracts

P-1. EFFECTS OF N-3 AND N-6 FATTY ACID PATHWAY INTERMEDIATES ON THE VIABILITY AND PHOSPHOLIPID MEMBRANE COMPOSITION OF HUMAN BREAST CANCER CELL LINES

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N-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been found to inhibit breast cancer growth. These fatty acids can be produced endogenously from α-linolenic acid (ALA); likewise, n-6 PUFAs can be produced from linoleic acid (LA). There is some evidence that pathway intermediates, γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), stearidonic acid (SDA) and eicosatetraenoic acid (ETA) may possess anti-carcinogenic properties. The purpose of this study was to investigate the effect of n-3 and n-6 pathway intermediates on the viability and phospholipid (PL) membrane composition of breast cancer cells. Tumorigenic MDA-MB-231 and MCF-7, and non-tumorigenic MCF-12A human breast cells were treated individually with 150 μM of an n-3 (ALA, SDA, ETA, EPA, or DHA) or n-6 (LA, GLA, DGLA, or arachidonic acid (AA)) fatty acid for 48 hr. Live cell counts were performed using the trypan blue exclusion method; and lipid PL extracted and separated by thin layer chromatography, methylated and analyzed by gas chromatography. Neither the n-6 nor n-3 fatty acids altered viability of MCF-12A non-tumorigenic cells. However, SDA, ETA, EPA, DHA, GLA and DGLA decreased viability of both cancer lines compared to control conditions (37-78%, $p<0.05$). ALA and LA resulted in a small decrease in viability in MCF-7 cells (73-78%, $p<0.05$) and AA decreased only MDA-MB-231 viability (71%, $p<0.05$). Fatty acid analysis revealed that most PUFAs were not further elongated or desaturated upon incorporation into PL membranes; except SDA and GLA which also altered the concentration of ETA and DGLA, respectively, in PL of both cancer lines. Long chain PUFAs, including pathway intermediates, appear to selectively reduce the viability (growth) of breast cancer cells and alter membrane PL by increasing PUFA content of that specific intermediate in PL. Anti-cancer properties of the n-3 and n-6 PUFAs do not appear to be due to their downstream conversion to pathway endpoints. (Funded by AVAC/AIBS and CIHR, H Yu is the recipient of a scholarship from the Canadian Dairy Commission)

Notes:

P-2. BINDING INTERACTION OF CYCLOLINOPEPTIDES TO HUMAN SERUM ALBUMIN

Youn Young Shim, Denis P. Okinyo-Owiti, Jianheng Shen, Pramodkumar D. Jadhav, and Martin J.T. Reaney
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Cyclolinopeptides (CLPs) are octa-, nona and deca-peptides present in flax flax (*Linum usitatissimum* L.) seed that may have antitumor activities but little is known of their pharmacokinetics. Human serum albumin (HSA), the most abundant protein in blood plasma, is an important mediator of flux of organic solutes and hence drug efficacy. Quantitative thermodynamic analysis of the interaction of compounds with HSA is important in the development of biomedical applications. Surface plasmon resonance (SPR) biosensors were utilized to reliably determine binding constants for several CLPs with HSA. All CLPs tested bound HSA with different affinities, with more than an order of magnitude difference in binding constants observed. For example, the maximum binding response of CLP-A/HSA was almost 20-fold higher than CLP-E/HSA. Through analysis of an array of peptides it was possible to correlate the impact of structural changes on CLP binding. The oxidation of sulfur in methionine residues to form methionine sulfoxide significantly reduced binding. Most strikingly, the further oxidation of methionine sulfoxide to the sulfone produced CLPs with strong binding. The large impact on binding by relatively small modifications of methionine containing CLPs suggests that hydrophobic interaction was the predominant intermolecular force stabilizing the complex between CLPs and HSA. The SPR binding studies may aid in the development of cyclic peptides as drugs or drug carriers.

Notes:

P-3. ASSOCIATION OF LIPID OXIDATION ASSAYS WITH TOCOPHEROL ISOMER ACTIVITY IN DIFFERENT OILS

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The oxidative pattern of 14 different oils with a wide range of unsaturation was determined using a battery of assays that measure lipid oxidation. Results were correlated with tocopherol (Toc) isomer content; specifically: α-Toc, γ-Toc, and δ-Toc. Lipid oxidation was measured using rancimat analysis (e.g. initiation phase), and by conjugated diene, triene and an iodometric assay (e.g. propagation phase). The third series of methods involved procedures that measured specific products of secondary lipid oxidation (e.g. TBARS), and other reactive decomposition products that included GSH oxidation, iodide and hemoglobin oxidation. A-Toc exhibited the strongest positive correlation with generation of conjugated diene ($R=0.607$, $p<0.05$) and triene ($R=0.746$, $p<0.05$) in unheated oil, suggesting pro-oxidant activity of α-Toc. On the other hand, α-Toc was inversely related to GSH oxidation ($R=-0.552$, $p<0.05$) by the aqueous extract of oil samples, indicating antioxidant activity of α-Toc. G-Toc exhibited similar, albeit a weaker relationship with conjugated triene ($R=0.578$, $p<0.05$). There was no significant relationship between δ-Toc levels and lipid oxidation end-point measures, except that the aqueous extract of the oil was positively correlated to the formation of methemoglobin ($R=0.650$, $p<0.05$) and iron-catalyzed iodide oxidation ($R=0.741$, $p<0.05$). Heat treatment of oils for 7 days at 95°C resulted in weaker relationship between Toc isomers and the different lipid oxidation product values. Differences in the relative anti-/pro-oxidant activity of Toc isomers can be attributed to the different partition coefficients for Tocs that favor solubility of the more hydrophobic α-Toc in bulk oil. In conclusion, measurement of lipid oxidation products or reactions that are specific to different stages of lipid oxidation in oils with characteristically different degree of unsaturation, proved to be useful in explaining the underlying mechanisms for Toc anti-/prooxidant activity.

Notes:

P-4. EFFECTS OF MILK ULTRAFILTRATION ON THE QUALITY OF DUTCH CHEESE WITH REDUCED FAT CONTENT DURING RIPENING

Michael Olkowski[1,2], Antoni Pluta[1], Anna Berthold-Pluta[1], Martin J. T. Reaney[2]
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Dutch Cheeses are classified as highly nutritious foodstuffs. Beside high quality protein and fat, Dutch Cheeses are good source of calcium, as well as many other important nutrients such as vitamins, essential microelements, bioactive peptides, probiotic bacteria, etc. Undesirable quality of Dutch-Cheese for some consumers may be relatively high caloric value, and industry offers a variety of products containing lower levels of fat. However, fat plays an essential role in functional and organoleptic characteristics of food, and therefore reduction of fat content may adversely affect textural and sensory properties. We examined various technological approaches to changes in nutritional, textural, and organoleptic factors of Dutch Cheese with reduced fat content. It was noted that ultrafiltration of the milk used in the production of Dutch Cheese resulted in better utilization of all milk proteins, and at the same time increased calcium level by approximately 20%, and reduced fat to 17.0% compared to the control samples. Ultrafiltration of milk during processing resulted in beneficial changes in the composition of whey proteins. This technological approach enhanced the buffering capacity of cheese and increased pH values, and these changes improved structural characteristics of cheese, as well as organoleptic properties.

Notes:

P-5. CHOLINE DEFICIENCY IMPAIRS INTESTINAL LIPID METABOLISM IN LACTATING RAT

Nusaibah Ab Aziz, Karen B. Kelly, Mélanie Légaré, Kelly-Ann Leonard, Neele Dellschaft, Sue Goruk, Jonathon Curtis, Donna Vine, Spencer Proctor, Catherine Field, René L. Jacobs
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Choline is an essential nutrient that is needed for brain development, lipid transport and organ function. Prolonged dietary deficiency can lead to hepatic, renal, pancreatic, memory and growth disorders. Even though humans can synthesize choline by the methylation of phosphatidylethanolamine to phosphatidylcholine (PC), catalyzed by the enzyme phosphatidylethanolamine *N*-methyltransferase, evidence exists that it may be insufficient to meet daily requirements. Pregnancy and lactation are situations when demand for dietary choline is especially high. The transport of choline from mother to fetus can deplete maternal stores of choline. Further, women with low dietary choline intake have a greater risk of giving birth to a child with a neural tube defect compared to those with higher-choline intake. To investigate the metabolic consequence of reduced choline supply we fed lactating dams either a Choline-Deficient (CD) or Choline-Supplemented (CS) diet for 20 days. Following lactation, the CD diet-fed dams weighed 10% less than those that ate the CS diet. Choline deficiency dramatically reduced plasma cholesterol, triacylglycerol (TG) and PC levels. To investigate the role of dietary choline in regulating intestinal lipid metabolism, we isolated jejunal scrapings from dams in both experimental groups. CD resulted in a reduction in the villus length of the small intestine and increased storage of cholesterol esters and TG. The build-up of neutral lipids may be explained by a reduction in postprandial TG appearance following an oral fat challenge. Analysis of intestinal gene expression may provide a mechanism for these observations. Taken together, it is clear that choline supply during lactation is vital for normal intestinal lipid metabolism.

Notes:

P-6. FLAX CYCLOLINOPEPTIDES ARE FREE RADICAL SCAVENGERS

Oyunchimeg Sharav[1],Peta-Gaye G. Burnett[2], Denis P. Okinyo-Owiti[2], Ramaswami Sammynaiken[3], Martin J. T. Reaney[2]
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Flaxseed oil, like other vegetable oils, contains non-triglyceride molecules that act as antioxidants. Freshly pressed flaxseed oil contains dissolved cyclic peptides (cyclolinopeptides or CLPs) that may have antioxidant activity. Even though CLPs elicit a wide range of biological effects, their main role in flax is not understood. Our previous investigation showed that addition of CLPs to peptide-free oil could improve oil oxidative stability. The CLPs may exert their effect through their free radical scavenging properties. CLPs A, B, C, G and G" were investigated employing electron paramagnetic resonance spectroscopy (EPR) measurements. The CLPs showed remarkable radical scavenging activity for removal of 1,1-diphenyl-2-2-picrylhydrazyl (DPPH) radical. Radical scavenging activity was dependent on CLP concentration and constituent amino acids as was observed from EPR measurements. Tryptophan-containing cyclolinopeptides, CLPs G and G", showed stronger scavenging activity than peptides that lacked this amino acid. Moreover, we observed that quenching of DPPH radical could be activated by UV irradiation especially for CLP A, B, and C. Novel reaction products between DPPH and methionine containing CLPs were detected by HPLC-MS analyses and were characterized by HPLC-MS/MS. The results of free radical scavenging activity of CLPs and possible mechanisms of radical scavenging will be presented and discussed.

Notes:

P-7. RUMINANT TRANS FAT TRANS-11 VACCENIC ACID IS MORE BIOAVAILABLE FROM A TRIGLYCERIDE SOURCE AND ACTS AS A NATURAL PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR LIGAND IN A RODENT MODEL OF DYSLIPIDEMIA AND METABOLIC SYNDROME

Ye Wang[1,2], M.Miriam Jacome-Sosa[1,2], Martin J. Reaney[3], Jianheng Shen[3], Shannon Scott[4], Catherine J. Field[2], Donna F. Vine[1,2] and S.D Proctor[1,2]
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Conjugated linoleic acid (*cis*-9, *trans*-11 CLA) and *trans*-11 vaccenic acid (VA) are natural *trans* fat present in ruminant-dairy foods. CLA has been shown to have numerous potential health related effects and has been extensively investigated. More recently, VA has been shown to have independent lipid-lowering properties associated with reduced lipid accumulation in the liver, as well as improving intestinal-associated dyslipidemia in animal models. Therefore, the aim of this study was to (i) assess VA as a natural PPAR-α/γ ligand *in-vitro* (ii) determine the effect of dietary VA on regulating intestinal mRNA and protein abundance of PPAR-α/γ *in-vivo* and (iii) compare the intestinal bioavailability of VA provided as a FFA and a TG source *in-vivo*, using an animal model of the metabolic syndrome (the JCR:LA-*cp* rat). The IC_{50} of several fatty acids to PPAR-α/γ ligand binding domain was assessed using a TR-FRET competitive binding assay. The resultant inhibition curves indicate that VA is a potent ligand to both nuclear receptors with lower IC_{50} compared to their respective synthetic agonists (indicative of greater binding capacity). *In-vivo*, the intestinal mRNA and protein expression of PPAR-γ were increased ($p<0.01$ and $p<0.05$, respectively) in hyperlipidemic rats fed 1.0% VA for 16 weeks, relative to control. In addition, VA from a TG source showed greater intestinal bioavailability assessed by mesenteric lymph sampling as compared to its free fatty acid form in hyperlipidemic rats ($p<0.01$). In conclusion, VA may possess greater PPAR-dependent binding *in-vitro* than some common therapeutic agents to treat hypertriglyceridemia (e.g. DHA and fenofibrate). The health benefits and greater bioavailability of VA from natural sources provides impetus for food labeling regulations to differentiate VA from industrially produced *trans* fat and warrants further investigation of its clinical implications under dyslipidemic conditions.

Notes:

P-8. VALIDATION OF A LC-MS/MS METHOD FOR THE QUANTIFICATION OF CHOLINE-RELATED COMPOUNDS AND PHOSPHOLIPIDS IN FOODS AND TISSUES

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A HILIC LC-MS/MS method was developed and validated to simultaneously quantify 6 choline-related compounds plus 8 phospholipid classes in a single run. The electrospray MS/MS method was optimized for each compound class so that the final method used a combination of precursor ion, fragment ion and neutral loss scans. Validation demonstrated that the method is sufficiently sensitive, and is accurate, precise and linear over 2 orders of magnitude. Recoveries in the range of 90% to 115% were obtained by spiking a range of sample matrices with authentic standards containing all of the target analytes. Accuracy and precision for quantification of phospholipid classes were also confirmed by ^{31}P NMR. A variety of sample matrices were analyzed using the validated method including local eggs, complete local diets recreated from eating records and rat organs from animal studies. Measured choline contents of foods were compared with values obtained using the USDA database.

Notes:

P-9. DOCOSAHEXAENOIC ACID ALTERS CELL DEATH, CANCER AND CELL-CYCLE SIGNALING NETWORKS IN BREAST CANCER CELL LINES

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Long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) are known to reduce viability of breast cancer cells *in vitro* and *in vivo*. While several mechanisms of action have been identified, a thorough investigation of differential gene expression induced by these fatty acids is lacking. The objective of this study was 9999999to measure alterations in gene expression in breast cancer cells treated with docosahexaenoic acid (DHA) and eicosapentaneoic acid (EPA). MDA-MB-231 (estrogen receptor, ER^-) and MCF-7 (ER^+) human breast cancer cell lines were treated with control medium, 100 μM linoleic acid (LA; n-6 fatty acid control), DHA or EPA for 48 h. Cellular mRNA was isolated, purified, labelled, fragmented, and hybridized to Affymetrix GeneChip Human Gene ST 1.0 microarray chips. Data were normalized, filtered for low-expressing genes and analyzed for differential gene expression (Partek Genomics Suite) and network analysis conducted (Ingenuity Pathway Analysis). Principal Component Analysis revealed that 40% of the alteration in gene expression was due to treatment in both cell lines. All fatty acid treatments changed networks of lipid metabolism and small molecule biochemistry, compared with control ($P<0.05$). DHA altered cancer, cell death and tumor morphology cellular signaling networks, compared with all other conditions, while EPA impacted an array of non-cancer related networks, including tissue development and organismal injury ($P<0.05$). A number of previously unidentified genes were differentially expressed by DHA treatment, including upregulation of RPS27, BAK1, IL1-α and SOD2, and down regulation of SPRY1, SOCS3, JAK2 and SMAD2. This study provides an understanding of the cellular and genomic changes induced by n-3 PUFA in breast cancer cells, and supports previous studies by our group and others that demonstrate a greater impact of DHA on breast cancer cell death compared with EPA. (Funded by CIHR and JBE funded by a CIHR postdoctoral fellowship).

Notes:

P-10. OBTAINING MILK FAT FRACTIONS ENRICHED IN CONJUGATED LINOLEIC ACID

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Conjugated linoleic acids (CLA) are positional and geometrical isomers of linoleic acid having a conjugated double-bond system. Studies suggest that CLA has numerous potential health benefits. Milk fat is the richest source of CLA in human diet and its concentration can be markedly enhanced through diet manipulation and nutritional management of dairy cows. Unfortunately, CLA is not stable upon thermal processing and significant losses in terms of biological activity occurred that might limit the applicability of CLA as a bioactive food ingredient. Further value-added products of processed milk fat are desired to generate new opportunities. In this study, anhydrous milk fat rich in CLA was enzymatically hydrolyzed and dry fractionated to obtain a milk fat fraction rich in CLA that can be used as an ingredient. The enzymatic hydrolysis was conducted at two different temperatures (50 and 70˚C) and different molar ratios of oil-water (70-140) and enzyme to oil ratio in the range of 40-80% (wt/wt). Additionally, the enzymatic hydrolysis was statistically optimized to yield more than 92% w/w of free fatty acid at 50ºC, 80% enzyme/oil (w/w) and 70.42 water/oil molar ratios in 24 hours. The hydrolyzed fat was oxidized using differential scanning calorimetry (DSC) at five different heating rates (3, 6, 9, 12, and 15˚C/min) in a temperature range of 100-350˚C. Then, the hydrolyzed fat was further dry fractionated to obtain three well defined milk fat fractions: (1) high melting point fraction from 25 to 12˚C, (2) medium fraction from 11 to 0˚C, and (3) low fraction from -26 to -38˚C. The fatty acid composition in each fraction was determined. Anhydrous milk fat from milk without enrichment was used as the control. Weight percentages of each fraction were 37.06%, 51.56% and 8.29% for high, medium and low melting point fractions, respectively. Around 3.71% corresponds to a fraction between high and low melting point fraction that was difficult to recover. The highest CLA concentration was found in the medium melting point fraction (64.89 mg CLA/g fat), which is importantly higher compared to those values obtained in the control fraction (5.9 mg CLA/g fat). Further work is needed for a detailed optimization of the fractionation protocol to maximize the CLA content of the medium fraction, minimizing the other fatty acids.

Notes:

P-11. SIMULTANEOUS OVER-EXPRESSING OF FATB AND SILENCING OF SADs BY ARTIFICIAL miRNAs INCREASES SATURATED FATTY ACID LEVELS IN *BRASSICA NAPUS* SEEDS

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Modifying fatty acid composition in plant seed oil is attracting more and more attention for its benefits to improve human nutrition and promote human health. To decrease the utilization of industrially hydrogenated vegetable oils *Brassica napus* cv. DH12075 was genetically engineered by simultaneous over-expressing of fatty acyl-ACP thioesterase B (FATB), a novel isolated FATB cDNA gene from developing seeds of *Brassica napus* cv. DH12075, and silencing of Stearoyl-ACP Desaturases (SADs) by artificial miRNAs to increase saturated fatty acid levels in seeds. Napin, a seed specific promoter, was used to restrict the changes of gene expression and fatty acid biosynthetic pathways within seed tissue. RT-PCR analysis showed that FatB gene was strongly over-expressed in six of B. napus transgenic lines compared to that of wide type DH12075, and the expression of BnSADs was significantly inhibited. The highest saturated fatty acids content in the seeds of line Bn48-93-5 was 48.4% by GC-FAMEs analysis. The Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis results showed palmitic acid (C16:0) and oleic acid (C18:1) are the predominant fatty acids in the transgenic Brassica napus seed oil, and the different triacylglycerol (TAG) compositions present, the major ones being PPO and POO. The melting point of the extracted seed oil was improved from -10 ^{o}C in DH12075 to 15.0 ^{o}C in Bn48-93-5. The extracted seed oil can be directly used for making healthy margarine and shortening etc. without the need of trans-fat-producing hydrogenation process. All the results indicated that simultaneous highly specific over-expressing of FATB and silencing of SADs by artificial miRNAs increased saturated fatty acid level in Brassica napus seeds significantly and effectively.

Notes:

P-12. EXPRESSION OF A NOVEL *ARABIDOPSIS THALIANA* PHOSPHOLIPASE A_2 cDNA IN YEAST

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The plant phospholipase A_2 (PLA_2) family is composed of multiple enzymes that catalyze the hydrolyzation of fatty acids from the *sn-2* position of phospholipids. PLA_2s have important roles in a wide range of physiological processes in plants including acyl remodeling, lipid synthesis, membrane homeostasis, stress responses, and cell signaling. In this study, we isolated a full-length *PLA_2* cDNA from *Arabidopsis thaliana,* linked the coding regions to the *GAL1*-inducible promoter in the yeast expression vector pYES2.1, and transformed into wild type *Saccharomyces cerevisiae*. The recombinant protein exhibited strong PLA_2 activity. The catalytic properties and substrate specificity of this enzyme are currently being characterized. .

Notes:

P-13. Isolation, Extraction and Purification of Cyclolinopeptides D", F" and G"

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Flax (*Linum usitatisssimum*) seed oil contains cyclic hydrophobic peptides or cyclolinopeptides (CLPs) containing eight or nine amino acid residues. To date, eleven CLPs (CLPs A to K) possessing molecular weights of approximately 1 kDa have been isolated and characterized from flaxseed and flaxseed oil. The CLPs (A, C, and E) have immunosuppressant activity and induce apoptosis in nematodes and a cancer cell line. Isolation and purification of individual peptides, not previously available, is a vital prerequisite for performing biological assays on these compounds. The recovery of individual hydrophobic peptides is important because each peptide may possess different biological activity and effect on gene expression in cells. Cyclolinopeptides are extracted from flax oil by adsorption onto silica gel, followed by recovery through solvent elution. The individual peptides are isolated from crude extracts using preparative reverse phase HPLC chromatography. The reduced forms of CLPs D, F and G (CLPs D", F" and G", respectively) were previously identified by MS/MS, but have never been isolated in sufficient quantities for complete characterization and/or biological activity assays. The present research has undertaken the isolation, extraction and purification of CLPs D", F" and G" in sufficient quantities. Complete characterization of these compounds via HPLC, LC-MS and NMR will be presented and discussed.

Notes:

P-14. FLAX CYCLIC PEPTIDES WITH FLUORESCENT TAGS

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Cyclolinopeptides (CLPs) are cyclic peptides present in flax seed oil comprising eight, nine or ten amino acid residues. To date, eleven CLPs (CLP-A to K), having molecular weights of approximately 1 kDa have been isolated from flaxseed oil, seeds and roots. Cyclolinopeptides are bioactive compounds with potential for use as therapeutics. CLPs (A, C, E) possess immunosuppressant activity and induce apoptosis in nematodes and a cancer cell line. CLP-C [*cyclo*(-Pro-Pro-Phe-Phe-Val-Ile-**Mso**-Leu-Ile-)] and CLP-E [*cyclo*(-Pro-Leu-Phe-Ile-**Mso**-Leu-Val-Phe-)] possess a methionine sulfoxide (**Mso**) that can be selectively modified by introducing activated groups (cyano (-CN) and ester (-COOEt)) without affecting the backbone structure or its configuration. The modified methionine groups in these CLPs can be used as synthetic tags to attach fluorescent labels for use in bioimaging and protein trafficking. In the present investigation, the –CN and –COOEt groups were introduced into CLPs (C and E) and were readily converted to $–NH_2$, –OH and –COOH groups, respectively. The chemically modified peptides were coupled with fluorescent labels including coumarin dye and their fluorescence emission spectra were recorded. These novel compounds were fully characterized with extensive 2D NMR methods: ^1H-^1H COSY, HSQC, NOESY, and HMBC. Complete synthesis and spectroscopic characterization of the novel peptides will be discussed.

Notes:

P-15. COMPARATIVE GENOME ANALYSIS REVEALS B3 TRANSCRIPTION FACTORS ARE PROMINENT IN BRASSICACEAE AND MAJOR CROP SPECIES

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The plant-specific B3 superfamily of transcription factors includes five important gene families, among which ABI3-VP1 (ABSCISIC ACID INSENSITIVE3 – VIVIPAROUS1) and ARF (Auxin Response Factor) members have been well studied, and ABI3-VP1 contains several vital regulators for the accumulation of seed storage reserves. This superfamily is characterized by the existence of one or multiple B3 domains, or the combination of the B3 domain and one or more of four additional domains including AP2 (APETALA2), ARF, Aux/IAA, and zf-CW (Zinc finger CW). Using a computational pipeline we developed for high-throughput domain discovery at a genome scale, we identified 94, 195, 58, 94, 81, 55, and 77 B3 transcription factors in the fully sequenced genome of Arabidopsis (*Arabidopsis thaliana*), *Brassica rapa*, castor bean (*Ricinus Communis*), cocoa (*Theobroma cacao*), soybean (*Glycine max*), maize (*Zea mays*), and rice (*Oryza sativa*), respectively. In relation to the estimated gene content in these genomes, the B3 superfamily has been significantly expanded during the evolution in eudicots, especially in the Brassicaceae lineage. We also observed further B3 domain duplication in some of these B3 proteins, forming more complex domain architectures than currently assumed. Structure prediction detected two α-helices and at least three β-sheets in most of these B3 domains, and this structural core is largely conserved despite their substantial sequence divergence. Expression analysis using public microarray data and expressed sequence tags revealed different expression patterns of *B3* genes in vegetative and reproductive tissues, suggesting their distinctive functions in different stages of plant growth and development. This study provides a framework for future investigations into potentially critical roles of *B3* genes in agriculturally important crops.

Notes:

P-16. IDENTIFICATION OF GENES INVOLVED IN LIPID BIOSYNTHESIS IN DEVELOPING SEEDS OF SEA BUCKTHORN (HIPPOPHAE RHAMNOIDES L.)

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Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, fruit-bearing shrub native to Asia and Europe that is gaining popularity as a nutraceutical plant. Sea buckthorn berries contain a variety of bioactive components as well as unique fatty acid profiles in the pulp and seed oils, which suggest differences in the lipid biosynthetic enzymes involved in pulp and seed oil biosynthesis. The goal of the current study was to characterize the lipid profiles of pulp and seed oils from Canadian-grown sea buckthorn cultivars and to identify sea buckthorn genes involved in lipid metabolism. Sea buckthorn berries were harvested from four cultivars (ssp. *mongolica*) at various stages of fruit development. Lipid analysis showed that in general, the pulp oil contains high levels of palmitic (16:0) and palmitoleic acid (16:1$^{cis\Delta 9}$), while the seed oil contains nearly equal amounts of linoleic (18:2$^{cis\Delta 9,12}$) and α-linolenic acid (18:3$^{cis\Delta 9,12,15}$), accounting for 60-70% of the seed oil composition. Using high-throughput 454 sequencing, most of the genes involved in fatty acid synthesis and glycerolipid assembly were identified from the transcriptome of developing seeds. *Stearoyl-ACP desaturase* (*SAD*), which catalyzes the formation of oleic acid(18:1$^{cis\Delta 9}$), had the highest number of reads, which was consistent with the high levels of oleic acid and its downstream products in sea buckthorn seed oil. Analysis of gene expression, fatty acid composition, and oil content at various developmental stages suggested that seed oil formation occurs in the early stages of fruit development and levels off following the green-yellow fruit stage, while pulp oil biosynthesis continues throughout the later stages of fruit development. Together, these results form a basis for further studies on lipid metabolism and for biotechnological improvement of sea buckthorn oil.

Notes:

P-17. PUTATIVE REGULATORY ROLE OF THE N-TERMINAL DOMAIN OF *BRASSICA NAPUS* DIACYLGLYCEROL ACYLTRANSFERASE 1 (DGAT1)

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Diacylglycerol acyltransferase (DGAT) catalyzes the final acylation step in triacylglycerol (TAG) synthesis and may exert substantial control on the overall flux of carbon into seed oil. In the present study, we report the identification and characterization of *DGAT1* genes in *Brassica napus*. Southern Blot hybridization of *B. napus* DH12075 genomic DNA with a fragment containing a conserved portion of *BnDGAT1* confirmed the presence of four *DGAT1* genes. These four genes possess a relatively high degree of polymorphism in the exon region encoding the hydrophilic N-terminus of the enzyme forms. The biological relevance of the variable N-terminal regions was further investigated using N-terminal deletions or domain swapping experiments between the N-terminal regions of two different BnDGAT1 isoforms. Chimeric *BnDGAT1* genes were transformed into yeast and microsomal extracts were tested for protein expression and enzyme activity. Results obtained from N-terminal deletion experiments suggested that this region is non-essential for catalysis. Interestingly, some of the chimeric enzymes with interchanged N-terminal domains produced twice as much oil in yeast as the native enzymes. Western Blots of protein extracts from these yeast cultures confirmed that the increased DGAT activity is largely due to increased enzyme accumulation, suggesting that the turnover of BnDGAT1 may be regulated by the amino acid modifications in its N-terminal region. Given the putative role of the N-terminus in regulating the quantity of DGAT produced and its potential impact on seed oil accumulation, we are presently testing targeted modifications of the N-terminus in an *Arabidopsis thaliana* mutant AS11 where *DGAT1* is inactivated.

Notes:

P-18. THE EFFECT OF OXYGEN AND LIGHT ON OIL PRODUCTION IN DEVELOPING *BRASSICA NAPUS* SEEDS

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Several research groups have demonstrated that environmental conditions, including oxygen and light, exert a substantial influence on seed oil production. It has been suggested that the seed coat affects oil accumulation in developing *Brassica s*eeds by serving as a permeability barrier to both light and oxygen. We hypothesized that yellow-seeded (YNO1-429) and black-seeded (DH12075) *B. napus* lines would respond differently to oxygen and light treatments due to their different seed coat properties, and that these differential responses would be reflected in seed oil production.

B. napus DH12075 and YN01-429 siliques (cotyledon stage) were exposed to 8, 21, or 40% (v/v) oxygen and varying light intensities (from 20 to 1500 µmol/m2/s) for 48 hours. To detect the small changes in lipid accumulation that result from the different oxygen and light treatments, the treated tissues were evaluated for diacylglycerol acyltransferase (DGAT) activity. This enzyme catalyzes the final step in triacylglycerol (TAG) production. At ambient oxygen (21%), no significant increase in DGAT activity was seen for either line upon increasing the light intensity from 20 to 1500 µmol/m2/s. At elevated oxygen (40%) and low light intensity, though, a significant increase in DGAT activity was observed for both DH12075 and YN01-429. Experiments were then performed to examine the impact of different oxygen and light conditions on the incorporation of radiolabelled acetate into TAG. Unlike the DGAT assay results, 14C-labelling revealed that light intensity, and not oxygen concentration, had the strongest impact on TAG accumulation in the developing seeds of B. napus. Possible explanations for this discrepancy will be presented.

Notes:

P-19. FIRST REPORT OF OUTCROSSING IN CAMELINA [*CAMELINA SATIVA* (L.) CRANTZ], A POTENTIAL PLATFORM FOR BIOINDUSTRIAL OILS

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Camelina [*Camelina sativa* (L.) Crantz] is a European old world oilseed crop of the Brassicaceae family being developed for cultivation in Canada. Presently camelina is being used as a feedstock for the production of biodiesel, however genetic engineering may expand its uses for production of bioindustrial oils. Due to the novel nature of this crop, outcrossing rates have yet to be defined for the western prairies. The objective of this study was to ascertain, under field conditions, the short distance intra-specific outcrossing rate for camelina. Results from this initial, small scale, fifteen replicate strip trial found camelina outcrossing rates to be less than 0.25%. Outcrossing in camelina was affected by direction, planting date and distance (20, 40 or 60cm) from the pollen source. Percentages of outcrossing were low (0.09 – 0.21%), suggesting camelina is a primarily a self-pollinated species. Quantification of pollen-mediated gene flow informs decisions about the suitability of the crop for genetic engineering applications as well as precautions such as isolation distances that may be required during variety development of transgenic crops. While pollen-mediated gene flow is low, it is only one avenue for adventitious presence. Other routes of gene flow may include seed-mediated gene flow and inter-specific gene flow to wild and weedy relatives. The detection of pollen-mediated gene flow, albeit low, in camelina demonstrates that stewardship and due diligence should be employed to mitigate transgene movement from genetically engineered camelina. Data from this study will be used to plan the appropriate size of medium field scale experiments to better characterize camelina outcrossing rates.

Notes:

P-20. RECOMBINANT EXPRESSION OF PLANT DIACYLGLYCEROL ACYLTRANSFERASES FROM SEA BUCKTHORN PULP AND COCOA BEAN

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Solid fat is required for many food products, including margarines, baking and frying goods. It is usually obtained from animal fats and tropical vegetable oils, as well as from hydrogenation of liquid unsaturated oils (primarily vegetable oils). However, during the hydrogenation process harmful *trans* fatty acids are generated. Vegetable oils enriched in saturated fatty acids (SFAs) could provide solid-fat functionality in food processing and be used as substitutes to reduce the degree of hydrogenation. Previous attempts to engineer oilseeds to accumulate SFAs targeted the early stages of fatty acid metabolism. Our focus is on latter steps of storage lipid assembly with particular attention to enzymes that can preferentially incorporate SFAs into triacylglycerol (TAG). In plants, TAG is the main seed storage lipid used to support the seedling growth after germination. Acyl-CoA:diacylglycerol acyltransferase (DGAT) catalyzes the final reaction in acyl-CoA dependent TAG biosynthesis, and is believed to exert control over the carbon flow into TAG. At least two types of DGATs are present in distinctive endoplasmic reticulum (ER) domains, and are designated DGAT1 and DGAT2. We isolated *DGAT* cDNAs from sea buckthorn (*Hippophae rhamnoides*) pulp and cocoa (*Theobroma cacao*) beans, both of which are rich in SFAs and monounsaturated fatty acids, and designated them as *HrDGATs* and *TcDGATs*, respectively. Expression of *HrDGAT1, HrDGAT2, TcDGAT1* and *TcDGAT2* restored TAG accumulation in the *Saccharomyces cerevisiae* mutant strain H1246, which is deficient in the DGAT activity and TAG accumulation. Yeast cultures expressing *DGAT1* displayed substantially higher accumulation of TAG compared to those expressing *DGAT2*. However, expression of *DGAT2* resulted in preferential synthesis of TAGs containing SFAs in yeast. Expression of *HrDGAT1* in developing seeds of *Arabidopsis thaliana* restored seed oil content and altered slightly the seed fatty acid composition with a significant reduction in the accumulation of eicosenoic acid ($20{:}1^{cis\Delta 11}$). These results suggest that *HrDGAT2* and *TcDGAT2* could potentially be used to increase SFA incorporation in vegetable oils. Combined with other strategies, this research might lead to vegetable oils with beneficial properties to the food industry.

Notes:

P-21. TRANSCRIPT PROFILING OF GENES INVOLVED IN TRIACYLGLYCEROL ASSEMBLY IN FLAX (*LINUM USITATISSIMUM* L.)

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Flax has been grown from ancient time mainly as source of oil and quality fiber. Flax seed oil is a rich source of α-linolenic (18:3 *cis*Δ9, 12, 15) acid, an essential dietary fatty acid that has numerous health benefits. Understanding the different biochemical processes involved in triacylglycerol (TAG) biosynthesis in flax is critical to improve the production and quality of flax seed oil. In the last decade, substantial progress has been achieved in the field of TAG biosynthesis with the discovery of several enzymes responsible for TAG assembly. The present study investigated the expression pattern of 16 genes encoding key enzymes directly involved in TAG assembly. The expression of each gene was monitored through quantitative real-time PCR assays using cDNA obtained from various tissues at different developmental stages. Transcript levels of genes encoding type-1 and type-2 acyl-CoA:diacylglycerol acyltransferases (DGAT), phospholipid:diacylglycerol acyltransferases (PDAT) and phosphatidylcholine:diacylglycerol cholinephosphotransferases (PDCT) were markedly higher in the embryo compared to vegetative tissues. Analysis of different developmental stages allowed us to categorize genes according to their expression profile. For example, genes encoding DGATs were highly expressed during mid to late stages of embryo development. Genes encoding PDATs appeared to have more stable expression throughout embryo development, while genes encoding PDCTs exhibited higher transcript levels during the early to mid stages of embryo development. These results point to a number of key genes which likely coordinate TAG biosynthesis in flax. Their temporal regulation suggests different physiological and biochemical roles in flax seed oil formation.

Notes:

P-22. INCREASING SEED OIL CONTENT THROUGH ENZYME ENGINEERING

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It is expected that the productivity of oleaginous crops will have to increase considerably to supply the vegetable oil demands for food and non-food applications. The economic benefit of such increase is obvious in canola, where a one percent increase in seed oil content could translate to an additional $90 million per year for the Canadian oil processing industry. But after decades of traditional breeding, the natural capacity of canola and other oilseeds to accumulate neutral lipids tends to plateau, requiring the support of novel technologies. Among different enzymatic steps involved in triacylglycerol (TAG) biosynthesis, the level of acyl-CoA:diacylglycerol acyltransferase (DGAT) activity appears to affect predominantly the flow of carbon into TAG. This was evidenced by over-expression of DGAT-encoding genes in oilseed crops. In the present research, our goal is to increase DGAT activity through enzyme engineering. Because the structures of DGATs are not understood in detail, we selected directed evolution through random mutagenesis as a method for enzyme improvement. To conduct directed evolution in DGAT, we developed an innovative high throughput screening method based on functional selection and fluorescence detection of TAG in a yeast recombinant system. With this method we were able to screen thousands of DGAT variants from mutagenized libraries that were created by error-prone PCR. After re-screening smaller subsets, we obtained variants displaying significant increases in yeast TAG accumulation. One variant with a single amino acid substitution produced a 7-fold increase in yeast TAG production compared to the unmodified native enzyme. This result was validated using site-directed mutagenesis, confirming that the observed increase in DGAT activity resulted from a single amino acid change. Through a continuous improvement of this and other DGAT variants, we expect to produce engineered enzymes that are highly active, express it in developing seeds, and contribute to seed oil production in canola and other oilseed crops.

Notes:

P-23. BIOACTIVE OILS PROGRAM – ADDING VALUE TO CANADA'S OILSEEDS

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Research on the nutritional effects of fats and oils in recent years has led to a decrease in the use of *trans* fats in foods and increasing acceptance and promotion of the health benefits of certain long-chain polyunsaturated fatty acids (PUFA) in the diet. The Bioactive Oils Program (BOP) represents an integrated approach to develop novel flax and canola seed oils to achieve these goals. The BOP team has a plant breeder working on the development of canola lines with increased saturated fatty acid content, complemented by molecular biologists endeavoring to improve the activity and selectivity of TAG-biosynthetic enzymes. A biotechnology approach is also being used to engineer flax seed with modified PUFA content. Coordinating with these activities, food processing researchers and analytical chemists have been focusing on the characterization and development of novel antioxidants and processing techniques in the context of maximizing the value of the newly developed oils. In addition, animal scientists will soon examine the efficacy of one of the more promising oils in aquaculture and laying hen applications. The BOP team also includes researchers focused on environmental risk assessment of new crops and social scientists identifying intellectual property barriers and examining issues such as consumer acceptability. As the program nears completion, several of the more promising BOP outcomes will be pursued and exploited through the newly established Alberta Innovates Bio Solutions Phytola Centre.

Notes:

P-24. NOVEL CYCLOLINOPEPTIDES IN FLAXSEED.

Peta-Gaye G. Burnett[1], Denis P. Okinyo-Owiti[1], Lester W. Young[1], Sandra Stone[2], Raju Datla[2], Michael K. Deyholos[3], and Martin J.T. Reaney[1]

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[3] Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada.

Cyclic peptides from plants are known to be highly biologically active materials with wide ranges of potential applications. Flaxseed (*Linum usitatissimum*) contains cyclic peptides comprising eight to ten amino acid residues. These cyclic peptides, referred to as cyclolinopeptides, induce a wide range of immunological responses in animals and cell cultures, including: (i) delayed-type hypersensitivity response; (ii) skin allograph rejection; (iii) graft versus host reaction; (iv) post-adjuvant arthritis; and (v) haemolytic anemia of New Zealand black (NZB) mice (Wieczorek *et al.*, 1991). Prior to this report, six cyclolinopeptides, having molecular weights of approximately 1 kDa have been isolated from flax roots, flaxseed and flaxseed oil. In our current investigation, the flax genome was screened via BLAST, PSIBLAST and RADAR for genes that might encode proteins that may be converted to cyclolinopeptides upon subsequent cleavage and cyclization. Our search yielded numerous candidate sequences of novel cyclolinopeptide-precursors, in addition to those that encode for previously known cyclolinoeptides. Consequently, flaxseed extracts were screened by HPLC-MS/MS for compounds with amino acid residues to determine if predicted nucleotide sequences were being expressed. We discovered five novel cyclolinopeptides in flaxseed and their corresponding putative gene sequences. The search strategies of the flax genome for potential peptide sequences and the mass fragmentation patterns of the new cyclolinopeptides will be presented and discussed.

Notes:

P-25 FERMENTATION OF STILLAGE

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Stillage, a by-product of the ethanol industry, is an aqueous mixture of organic and inorganic molecules. Typical organic constituents include glycerol, 1,3-propanediol, glycerolphosphorylcholine (GPC), betaine phosphate, and acetic acid. These compounds are potentially valuable without modification or as precursors for additional processing. Therefore, stillage is a potential resource for the production and recovery of value-added compounds. Here we report the modification of compounds present in stillage by bacterial fermentation. It was observed that storing stillage solution at 32°C led to conversion of the glycerol in the stillage to 1,3-propanediol. Interestingly, when strains of Lactobacillus bacteria were isolated from the fermenting stillage solution, enriched, and reintroduced to the stillage improved conversion of glycerol to 1,3-propanediol was observed. The conversion of waste glycerol from biodiesel and ethanol production to 1,3-propanediol will be discussed.

Notes:

P-26. DEVELOPMENT OF SODIUM ALKOXIDE CATALYSTS FROM POLYOLS FOR BIODIESEL PRODUCTION

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Metal alkoxide and hydroxides are popular and inexpensive base catalysts used by industry to produce fatty acid esters. Alkoxides are more expensive, their production is hazardous and they are dangerous to transport. The alkoxide produces higher yields of product and is often a preferred choice over hydroxide. In this study, low cost sodium alkoxide base catalysts were synthesized from 50 wt% sodium hydroxide solution and non-volatile, non-toxic polyols using evaporation which is less expensive and hazardous. Gravimetric analysis showed that polyols effectively aid in evaporation of 50 wt% aqueous sodium hydroxide during formation of alkoxide compounds. The resulting products are base compounds that were characterized using X-ray diffraction and elemental analyses. Results have shown that the polyol-derived alkoxide compounds are predominately mono-sodium substituted alkoxide that occur as adducts with sodium hydroxide. Studies of transesterification reactions catalyzed by polyol-derived sodium alkoxide/hydroxide were conducted to evaluate reaction efficiency and kinetics. The reactions catalyzed by the polyol-derived sodium alkoxide/hydroxide successfully achieved comparable biodiesel yield with sodium methoxide. Additionally, all polyol-derived alkoxide/hydroxide catalysts investigated in these studies were capable of achieving >95 wt% of biodiesel yield after 1.5 h. In conclusion, the polyol-derived sodium alkoxide/hydroxide catalysts have demonstrated promising quality to the industry. These catalysts may serve as an alternative solution to lower the cost of biodiesel plant operation without compromising production efficiency.

Notes:

P-27. PRODUCTION OF BIOLUBRICANT (TRIMETHYLOLPROPANE ESTERS) FROM FISH STEARIN

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The objective of this research was to optimize the production technology of bio-lubricant based on the use of fish stearin as raw material for the transesterification of fatty acid methyl esters (FAME) and trimethylpropane (TMP) bio-lubricant. FAME production was achieved by esterification of fish stearin with methanol in the presence of KOH and at a high reaction temperature. Production of trimethylolpropane esters (bio-lubricant) from FAME was achieved by reaction in a reversed phase system. Besides catalytic activity, other reaction parameters that could affect the conversion of FAME to trimethylolpropane esters (bio-lubricant) during the transesterification reaction, including reaction temperature (118 ^{o}C, 128 ^{o}C, 138 ^{o}C, 148 ^{o}C or 158 ^{o}C), the mole ratio of FAME to TMP (3, 3.5, 4, 4.5, 5 or 5.5), the mole ratio of catalyst to TMP (0.025, 0.05, 0.075, 0.1 or 0.15), and the reaction time (1 h, 1.5 h, 2 h, 3 h, 3.5 h, 4.5 h or 5 h), were investigated in a series of single factor experiments to obtain the optimum reaction conditions for producing bio-lubricant. Following gas chromatography analysis of the final trimethylpropane ester products, the optimum reaction conditions were determined to be: reaction temperature = 128 ^{o}C, mole ratio of FAME to TMP = 4, mole ratio of catalyst to TMP = 0.1, and reaction time = 1 hour.

Notes:

P-28. AN INVESTIGATION INTO THE OZONOLYSIS OF SIMPLE LIPIDS BY LC/MS AND GC/MS

Chenxing Sun, Yuanyuan Zhao, and Jonathan M. Curtis
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Products and intermediates of ozonolysis on methyl oleate (MO) and triolein (OOO) were studied by liquid chromatography - mass spectrometry (LC/MS), gas chromatography-mass spectrometry (GC/MS) and size exclusion chromatography with an evaporative light scattering detector (SEC/ELSD). As expected, the degree of unsaturation of both MO and OOO was shown to decrease gradually with an increase of ozonolysis time. However, the LC/MS data for MO also showed that the formation of trioxolanes reaches the highest yield after a certain period of ozonolysis beyond which time the trioxolanes concentration decreases. GC/MS also shows that the formation of light fraction products follows a similar trend. By LC/MS monitoring of the ozonolysis of OOO, it was possible to observe the formation of first the mono-ozonide, followed by the formation of di-ozonide and finally tri-ozonide. Eventually, all of the mono-ozonide and di-ozonide were seen to be converted to tri-ozonide. The observed decrease in tri-ozonide at still longer ozonolysis times is believed to be a result of oligomer formation, which is supported by the observation of higher molar mass species by SEC/ELSD. Understanding the progress of these reactions is important for the development of new processes making use of the ozonolysis of fats and oils.

Notes:

P-29. THE USE OF NON-AQUEOUS REVERSED PHASED LC/MS TO MONITOR THE FORMATION OF INTERMEDIATES AND THE REACTION KINETICS DURING THE EPOXIDATION OF CANOLA OIL

Tuan Nurul Sabiqah Tuan Anuar, Yuan-Yuan Zhao, Guoguong Liu, Jonathan M. Curtis
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This study was designed to monitor the formation of reaction intermediates during the epoxidation of canola oil. The epoxidation was performed by reaction with formic acid and hydrogen peroxide at 35°C for 28 hours. The intermediate products from the reaction were collected every hour to observe the epoxidation profile of canola oil triacyglycerols with 54 carbons. A simple and fast analytical method by means of non-aqueous reversed phase liquid chromatography coupled to electrospray mass spectrometry was developed in order to study the formation and disappearance of the partially epoxidised components. By monitoring the ratio of fully to partially oxidized products in the mass spectra. It was found that the end point of the reaction could be easily observed. In addition, the kinetic data is important for pilot scale process optimization.

Notes:

P-30. CHARACTERIZATION OF BYPRODUCTS FORMED FROM PILOT SCALE CANOLA OIL EPOXIDATION BY LC/MS/MS

Yuan-Yuan Zhao, Guoguang Liu, Ereddad Kharraz, Tolibjon Omonov, Paul Tiege, and Jonathan M. Curtis
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This study reports on the formation and characterization of byproducts found in the pilot scale epoxidation of canola oil by performic acid. From the accurate mass and MS/MS data, the possible structure and mechanism for formation of these formate alcohols due to acylation of epoxy groups by formic acid, were investigated. The results were confirmed by the reaction of oil epoxides with formic acid at different temperatures. Knowledge of the formation of the formate alcohols allowed us to modify the reaction conditions so as to avoid their occurrence during the pilot scale canola oil epoxidation process.

Notes:

P-31. BIODIESEL PROCESS DEVELOPMENT FOR INDUSTRIAL APPLICATIONS: ALTERNATIVE FEEDSTOCKS, EMERGING GREEN TECHNOLOGIES, AND QUALITY CHALLENGES

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The world is facing its worst energy crisis in history. Canada consumes ~23 million tons (~26 billion litres) of diesel annually, and the global consumption is 934 million tons of diesel fuel per year. The development of energy-efficient biofuel production technologies aiming at reducing the reagent costs and increasing the production efficiency are urgently needed to reduce energy consumption and to increase the process economics. Due to the high cost of refined vegetable oil, currently, biodiesel industry has moved towards using alternative inexpensive feedstocks with high free fatty acids (FFA). However, using traditional homogeneous base-catalyzed industrial process could result in higher acid number, yield losses, higher post-cleaning processes, lower quality of glycerin, product inconsistency and process inconsistency. In the past decade, many industrial processes were shifted toward using solid acid catalysts. Solid acid catalysts are generally preferred for chemical transformations in industrial processes because of their ease in separation from a reaction mixture. In addition, solid acid catalysts can potentially be regenerated, and they are environmentally benign because they can be reused and safe for industrial operations and will produce high quality glycerin (a byproduct). Because of these advantages, innovative solid acid-catalyzed processes are gaining attraction for the production of biodiesel. Currently, Canada produces about 200 million litres of biodiesel annually. It will need about 550 million litres of biodiesel to meet government mandate. As a result, new biodiesel industrial plants are under construction throughout Canada. In this presentation, an overview of biodiesel process development for industrial-scale production of biodiesel will be discussed including new alternative feedstocks, emerging green technologies, and challenges for meeting the biodiesel quality standards.

Notes:

P-32. A SIMPLE AND GREEN ANALYTICAL METHOD FOR THE DETERMINATION OF ACID NUMBER OF BIODIESEL AND BIODIESEL BLENDS USING GREEN CHEMISTRY APPROACHES

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Biodiesel, as an alternative green fuel, has gained importance throughout the world and has resulted in a significant increase in the commercial use of biodiesel and biodiesel blends. Due to the high cost of refined vegetable oils, alternative inexpensive feedstocks with high FFA content are gaining momentum around the world for the production of biodiesel due to its economical, commercial, and environmental benefits. This requires an accurate determination of acid number (AN) to monitor the progress of the biodiesel production process. As a result, the AN has been included in various quality standards including ASTM D 6751 and EN 14214 as an important quality parameter. ASTM D 664 is a reference method for measuring the AN of biodiesel and biodiesel blends in ASTM D 6751. The ASTM reference standard method D 664, has major problems such as use of excess toxic solvents, large sample size, mediocre reproducibility, tedious process for cleaning electrodes, and relatively long analysis time. It was found that determination of AN of biodiesel and biodiesel blend using the current ASTM D 664 results in large values of repeatability and larger % error when the sample size was reduced. Therefore, a new proposed method, based on green chemistry approaches, has been developed to determine the acid number of biodiesel and biodiesel blends using small sample size and reduced toxic titration solvent. Furthermore, a simple and efficient electrode cleaning procedure was developed. This makes the proposed method a green analytical method which is technically feasible, economically reasonable, and environmentally friendly. The application of this proposed method for the determination of AN of biodiesel and biodiesel blends was studied. This proposed green analytical method could be used for the determination of AN of biodiesel and biodiesel blends in R&D and industrial quality control laboratories as a simple, time-efficient, cost effective, and environmentally friendly method.

Notes:

P-33. A DIRECT METHOD FOR THE SYNTHESIS OF FATTY ACID METHYL ESTER (FAME) FROM CRUDE JATROPHA OIL AS SECOND GENERATION FEEDSTOCK USING VERSATILE GREEN CATALYSTS

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Due to the high cost of edible oils and growing concern of food for oil, the commercial production of biodiesel is in great need of new inexpensive alternative second-generation feedstocks which do not compete with food. Recently, Jatropha oil has been considered as one of the most promising potential feedstock for the production of biodiesel in Asia, Africa, Europe, South America, and now is gaining momentum in North America due to its advantages over edible oil such as it does not compete with food, high oil content, grown on marginal lands, inexpensive, and desirable valuable properties such as good stability, low viscosity, better cold properties, high cetane number and good low temperature properties. Currently, most of the biodiesel from Jatropha oil is produced by using a multi-step process with homogeneous catalysts which required complex downstream neutralization, separation, and washing steps which make the purification of the biodiesel more challenging. There is a great need for the development of a simple single-step solid acid-catalyzed process which is technically feasible, environmentally green, and economically reasonable. Therefore, in this research study, in continuation of our work to develop new solid acid-catalyzed processes for biodiesel production, herein, we are reporting a direct method for the synthesis of fatty acid methyl ester (FAME) from crude Jatropha oil, a second generation feedstock, using versatile solid acid catalysts. Also, as a part of process development, major process parameters for biodiesel production were investigated. These preliminary results are very promising and suggest the feasibility of using Jatropha oil as a low cost non-edible feedstock for the industrial production of biodiesel using a new solid acid single-step process.To the best of our knowledge, this is the first report on the development of a single-step solid acid-catalyzed process for the synthesis of FAME from crude Jatropha oil without any pre-treatment.

Notes:

P-34. ENZYMATIC CONVERSION OF CORN OIL TO BIODIESEL IN SUPERCRITICAL CARBON DIOXIDE

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Biosynthesis in supercritical fluids is a relatively new “green” technology attracting much attention. One promising application is the enzymatic production of biodiesel (fatty acid methyl esters, FAMEs) in supercritical carbon dioxide (SC-CO_2). In this study, synthesis of biodiesel from corn oil was studied in a batch SC-CO_2 reactor using immobilized lipase (Novozym 435) as catalyst. Effects of reaction conditions on the content of FAMEs, monoacylglycerols (MAGs), diacylglycerols (DAGs), and triacyglycerols (TAGs) were investigated at various enzyme loads (5-15%), temperatures (40-60 °C), substrate mole ratios (corn oil:methanol; 1:3-1:9), pressures (10-30 MPa), and times (1-8 h). The highest FAME content (81.3%) was obtained at 15% enzyme load, 60 °C, 1:6 substrate mole ratio, and 10 MPa in 4 h. A reaction kinetic model was used to describe the system, and the activation energy of the system was calculated as 89.5 kJ/mol. Elimination of the use of organic solvents, chemical catalysts and wastewater, and reasonable high yields make the enzymatic synthesis of biodiesel in SC-CO_2 a promising “green” alternative to conventional biodiesel process.

Notes:

P-35. THE EFFECT OF GROWTH MEDIUM PH ON OIL ACCUMULATION IN SEVERAL ISOLATES OF *CHLORELLA* NATIVE TO THE CANADIAN PRAIRIES AND THE ATHABASCA OIL SANDS

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The characteristics of biodiesel derived from microalgae are dependent on many things, such as the specific strain(s) of algae used, and the conditions under which it is grown. Nutrient stress has been the most common method of increasing algal oil yield; however it is technically challenging to exert fine control over the level of individual nutrients in a batch culture. Interestingly, reports from the literature suggest that growth medium pH can alter lipid accumulation in *Chlorella* and members of the Chlorophyceae. To discover if pH manipulation is a viable alternative to nutrient stress for the induction of oil accumulation in green algae, we compared its effects on lipid accumulation to that of nitrogen and sulfur deficiency in five isolates of *Chlorella*. Isolates were collected from soil and water samples in the Canadian prairies and identified based on morphological and molecular markers. Fatty acid profiles and TAG amounts were measured qualitatively by Nile Red fluorescence and quantified by gas chromatography. We found that overall lipid accumulation due to pH stress is equal to or greater than that caused by nutrient deficiency in most of our isolates. This was especially true when the pH was incrementally adjusted throughout the experiment; we also observed growth numbers equal to or exceeding those of unstressed cultures for this condition while still maintaining high oil yield. Our data confirm that the strain of algae used is very important in optimizing oil production, but we also demonstrate that by manipulating pH we can meet or exceed the oil content achieved with nutrient stress.

Notes:

P-36. DEVELOPMENT OF A CONTINUOUS CRYSTALLIZATION PROCESS FOR THE CONCENTRATION OF OMEGA-3 FATTY ACIDS FROM MACKEREL OIL

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Omega-3 polyunsaturated fatty acids (PUFA) are known to act against cardiovascular, inflammatory and autoimmune diseases, cancer and they are also considered necessary for normal growth and development of the brain and retina throughout the life cycle. These fatty acids cannot be synthesized by the human body, and hence must be obtained through diet. However, the population of today consumes much less amounts of omega-3 fatty acids through diet. Hence, there is a need of omega-3 PUFA concentrates, devoid of saturated fatty acids and monounsaturated fatty acids, as these are considered more beneficial than marine oils as they ensure low daily intake of total lipids.

Urea adduct formation was used for the concentration of omega-3 polyunsaturated fatty acids from mackerel oil. It involves the use of urea, which can selectively form complexes with only straight chain saturated fatty acids. In this process, the crystallization time, crystallization temperature and fatty acid to urea ratio were optimized. Previously, very long residence times in the crystallizer have been used for this process, usually being more than twelve hours. We were able to achieve 72% concentration of omega-3 PUFAs by single step urea adduct crystallization in thirty minutes. Mackerel oil was first converted to free fatty acids using chemical hydrolysis, and then subjected to urea adduct crystallization. The fatty acid composition was analyzed using GC. The optimum fatty acid to urea to solvent ratio was found to be in the range of 1:2:10 to 1:3:10 at a crystallization temperature of -15°C. The next objective will be to proceed towards making the process a continuous one by using kinetic data from the batch crystallizer to design a continuous crystallizer. The concentrate could be fractionated again to obtain highly concentrated omega-3 fatty acid fraction.

Notes:

P-37. $SCCO_2$ AND LIGNANS FROM FLAXSEED: EXTRACTION YIELD AND EFFECT OF SEED PRETREATMENT

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The effects of pretreatment on the supercritical CO_2 ($SCCO_2$) and traditional extractions of lignan secoisolariciresinol diglucoside (SDG) from flaxseed were investigated. Flaxseed defatted with petroleum ether and $SCCO_2$, full-fat hulls, defatted hulls and pre-hydrolyzed flaxseed were processed at the conditions (7.77 mol% ethanol, 45 MPa and 60 ^oC) which produced the maximum CO_2 loading of SDG. Residue and raw material samples were subjected to traditional lignan extraction. All samples were analyzed for SDG content using RP-HPLC. Cellular structure of samples before and after $SCCO_2$ extraction was examined using SEM to determine the effect of $SCCO_2$ processing on traditional lignan extraction. A colorimeter was used to determine if processing and SDG extraction have any effect on flaxseed colour. All samples, except the pre-hydrolyzed seeds, showed increases in traditional extraction efficiency post-$SCCO_2$ processing, likely due to the resulting changes in the flax matrix, as revealed in SEM images. $SCCO_2$ has potential benefits in enhancement of traditional solvent extraction of bioactives from plant materials.

Notes:

P-38. MINIMAL REFINING OF CANOLA OIL: EFFECTS ON PHYTOSTEROLS AND TOCOPHEROLS

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Canada is one of the major canola oil producers and the main exporter of canola oil in the world. It has shown that canola oil contains the lowest amount of saturated fatty acids and is a good source of tocopherols and free and esterified sterols compared to other vegetable oils. Currently, most part of extracted canola oil is refined by traditional methods. Traditional refining (degumming, neutralization, bleaching and deodorization processes) leads to the removal of about 40% of phytosterols and tocopherols found in canola oil.

Although, minor components have some effects on the nutritional properties, quality and stability of oils but, undesirable components may cause darkening, foaming, smoking, precipitation, development of off-flavours, decreased thermal and oxidative stability. Therefore, some of these undesirable minor components such as free fatty acids, chlorophyll, phosphatides, trace metals, oxidized products and pesticide residues should be removed from canola oil by refining methods. Unfortunately, some health promotion minor components such as sterols and tocopherols inevitably are removed from the oil during various stages of refining.

A Minimal Refining for Health (MRH) refers to the removal of undesirable components with the lowest impact on nutrients such as phytosterols and tocopherols found in canola oil. Clinical studies have shown that phytosterols lead to a reduction in bad cholesterol and also, some part of tocopherols act as natural antioxidants in the body. The MRH method is proposed based on using a weaker alkali for oil deacidification and different clays for absorption of impurities at mild processing conditions.In MRH method, sodium hydroxide is replaced with calcium hydroxide, sodium silicate or magnesium oxide for minimizing degradation of tocopherols during deacidification. Moreover, natural clays such as diatomaceous earth, Magnesol® and Trysil® are used for bleaching instead of acidified clays. The deodorization step is omitted in the MRH method to preserve natural canola oil flavour and important healthy components.

Minimal refining also has a smaller environmental impact compared to conventional refining methods because the hot water washing step that produces waste water during conventional refining is replaced by clays that absorb the soap and other impurities.

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Notes:

P-39. OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF SACHA INCHI OIL

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Sacha inchi has been recently commercialized for its high PUFA content unsaturated fatty acids, being the major component α-linolenic acid (a type of omega-3 fatty acid). Hydrolysis of seed oils is an important industrial unit operation to obtain desirable free fatty acids, which can be used as ingredients for enrichment of food or the production of structured lipids. The effect of temperature (40-60ºC), oil:water molar ratio (1:5-1:70) and, enzyme concentration (5-40% weight of oil) were evaluated using response surface methodology and Lipozyme TL IM for the conventional enzymatic hydrolysis of sacha inchi oil. Temperature and oil:water ratio did not show any effect on the degree of enzymatic hydrolysis of sacha inchi oil. In contrast, enzyme concentration had an important effect on percentage of free fatty acid production. The maximum hydrolysis (68.40 ± 0.98%) was achieved at 60ºC, oil:water molar ratio of 1:70 and 26.17% of enzyme concentration (weight of oil).

Notes:

P-40. HIGH THROUGHPUT HPLC ANALYSIS OF CYCLOLINOPEPTIDES IN FLAX SEEDS

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Cyclolinopeptides (CLPs) are small cyclic peptides found in flax seed oil. The world flax collection has over 2000 entries; therefore, effective analysis of these lines will require the development of new methods for high throughput screening. Recently, we have also discovered many new peptides, which has led to a revision of our previously published extraction and quantification methods. We report a high throughput liquid chromatography (LC-UV) method for quantification of trace amounts of CLPs (A, C, D, E, and F) from flax seed. The high throughput screening was achieved by simplifying sample preparation procedures and development of improved rapid LC analyses. Monolithic HPLC media (Chromolith® SpeedROD monolithic column 4.6 x 50 mm) were employed to reduce the analysis time per sample.

In order to quantify the amounts of CLPs, calibration curves were obtained using standard addition method to account for recovery and matrix effects due to the absence of a blank matrix. The gradient elution system was optimized, leading to improved detection limits for CLPs while achieving baseline separation. The sample preparation and high throughput analysis of CLPs will be presented and discussed.

Notes:

P-41. NOVEL POLYSACCHARIDE AEROGELS USING SUPERCRITICAL CARBON DIOXIDE: APPLICATION AS FLAXSEED BIOACTIVE CARRIERS

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Flax bioactives, including α-linolenic acid and secoisolariciresinol diglucoside (SDG) have been associated with various health benefits. Polysaccharide aerogels, dried using supercritical carbon dioxide ($SCCO_2$), have low densities and high porosities, making them ideal as bioactive carriers. Five % barley β-glucan and 10% flax mucilage hydrogels were dried using $SCCO_2$, and their characteristics analyzed. SDG concentrate was incorporated into the gels prior to drying using several techniques. SDG content was quantified using HPLC. Both aerogels had low densities; however, mucilage had higher surface area and retained a higher percentage of the original hydrogel volume compared to β-glucan, with 201.13 m^2/g and 56.90% compared to 165.55 m^2/g and 37.62%, respectively. When SDG concentrate was added to the hydrated polymer mixtures, regardless of technique, SDG contents were similar; however, less than half of the expected amount was recovered. Polysaccharide $SCCO_2$ dried aerogels, including those formed from flax derived polymers, show promise for use as a delivery vehicle for nutraceuticals, including flax SDG.

Notes:

P-42. PHYSICAL PROPERTIES OF CARBON DIOXIDE-EXPANDED CANOLA OIL AND ITS BLEND WITH CANOLA STEARIN

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Physical properties of lipid-type substances in equilibrium with high pressure carbon dioxide are influenced by CO_2 solubility in the liquid lipid phase, which increases with pressure. The experimental data obtained for CO_2 solubility in canola oil at 40 and 70°C and its blend with canola stearin (30 wt%) at 70°C up to 25 MPa were successfully correlated using engineering models involving equations of state. Volumetric expansion of canola oil at 40°C and 70°C and its blend with canola stearin at 70°C and pressures of up to 25 MPa in equilibrium with high pressure CO_2 was 41, 46, and 43%, respectively. The densities of canola oil at 40, 55, and 70°C and its blend with canola stearin at 70°C in equilibrium with high pressure CO_2 were measured using a view cell equipped with a spring balance based on Archimedes principle at pressures of up to 30 MPa and shown to increase to 4.71, 4.30, 3.52 and 3.57% of its value at atmospheric pressure, respectively. The density of CO_2-expanded lipids increased with pressure and decreased with temperature. Physical properties of CO_2-saturated lipids are important for designing high pressure processes and especially for better understanding of enzymatic transesterification of canola oil and canola stearin using supercritical CO_2 to produce base-stock for zero-*trans* margarines, where these physical properties influence the reaction rates due to their effect on mass transfer.

Notes:

Index by Author

Acharya, S.	14
Akin, O.	44
Angers, P.	17,95
Arul, J.	17,95
Asomaning, J.	35
Aziz, N. A.	64
Bagonluri, M.	16,27,99
Baig, A.	36,90,91,92
Bandara, M.	14
Banik, M.	21
Boothe, J. G.	24
Bressler, D. C.	35
Brett, N.	48
Budge, S.M.	46
Burnett, P. G.	16,65,72,73,83,99
Carlsson, A. S.	71
Chen, G.	71
Ciftci, O. N.	93
Clark, K. R.	23
Cloutier, S.	21
Comin, L.	96,100
Curtis, J. M.	15,33,37,64,67,87,88,89
Datla, R.	83
Dellschaft, N.	64
Deng, W.	76
Deyholos, M. K.	83
Dorit, A.	45
Downey, K.	9
Duguid, S.	21
Elisia, I.	62
Ewaschuk, J. B.	11,60,68
Fatima, T.	75,79
Field, C. J.	11,28,60,64,66,67,68
Fowles, R.	48
Friel, J.	45
Geerkens, C.	48
Ghazani, S. M.	98
Goruk, S.	64,67

Gray, M. R.	35
Greer, M. S.	76
Gui , B.	27,99
Gunenc, A.	18
Haakensen, M.	84
Hall, J.	46
Hall, L. M.	78,82
Harry, I.	24
Hazendonk, P.	47
He, Y.	23
Hills, M. J.	78
Hofmann, C.	48
Hosseinian, F.	18
Hryniuk, R.	14
Huidrom, D.	45
Jacobs, R. L.	64,67,68
Jacome-Sosa, M. M.	28,66
Jadhav, P. D.	61,73
Jadhav, V. P.	72
Jenab, E.	101
Kazachkov, M.	25
Kazala, C.	14,82
Kelly, K. B.	64
Khan, M.	69
Kharraz, E.	33,89
Kitts, D. D.	62
Kleppinger-Sparace, K. F.	23
Kong, X.	37
Krishna, P.	75,79
Lager, I.	71
Lawson, S. L.	77
Lee, E. L.	14
Légaré, M.	64
Leonard, K. A.	64
Liu, G.	33,37,88,89
Lopes-Lutz, D.	48
MacDougall, K. M.	38
Marangoni, A. G.	97
Martínez-Monteagudo, S. I.	43,69
Mazurak, V.	60

McGinn, P. J.	38
McKeon, T.	31
McNichol, J.	38
Melanson, J. E.	38
Moloney, M. M.	24
Mussone, P.	35
Nelson, R.	68
Newell, M.	11,60,68
Ng, F. T. T.	36,90,91,92
Nie,J .	34,86
Nykiforuk, C. L.	24
Oilund, K.	67
Oinam, G. S.	24
Okinyo-Owiti, D.	16,61,65,72,73,83,99
Olivia, C.	16,99
Olkowski, M.	63
Omonov, T. S.	33,89
Paszti, M. D.	91
Peng, F. Y.	74
Pluta, A. B.	63
Pluta, A.	63
Prado, G. H. C.	98
Prasad, R.	14
Proctor, S. D.	28,64,66
Przybylski, R.	47,82
Puttick, D. M.	78
Qiu, X.	82
Rahman, H.	26,82
Ratanapariyanuch, K.	84
Reaney, M.	16,27,28,34,41,61,63,65,66,72,73,83,84,85,86, 99
Reddy, N.	45
Reed, C.	24
Riley, W. W.	86
Rowland, G.	21
Ryan, R.	13
Saldaña, M. D. A.	43,69,96,98,100
Sammynaiken, R.	65,73
Scanlon, M.	45
Schatte, G.	85
Schieber,A.	48,82

Scott, S.	66
Shah, S.	82
Sharav, O.	65
Sharma, N.	76,77
Shen, J.	28,34,61,66,72,73,86
Shen, W.	25
Shim, Y. Y.	61,84
Siloto, R. M. P.	79,80,81
Singh, A.	15
Sinha, P.	95
Skrupski, B.	94
Smith, M.	70,82
Smyth, S.	82
Snyder, C.	14,71,75
Sosinska, E.	47
Sparace, S. A.	23
Stone, S.	83
Stymne, S.	71
Sun, C.	87
Sun, J. Y.	70
Tekle, T.	37
Temelli, F.	44,69,82,93,96,100,101
Thambugala, D.	21
Thiyam, U.	45
Thomas, J. E.	14
Tiege, P.	89
Topinka, K. C.	78
Tosta, C.	48
Truksa, M.	76
Tuan Anuar, T. N. S.	88
Vine, D. F.	28,64,66
Walsh, K. D.	78
Wang, L.	25
Wang, Ye.	66
Wang, Yo.	34, 86
Weselake, R.	14,60,71,74,75,76,77,79,80,81,82
Wickramarathna, A. D.	80
Wilson, K.	94
Xiong, Y.	67
Yang, R. C.	78

Yilmaz, J. L.	71
Young, J. W.	62
Young, L. W.	83
Young, T. E.	23
Yu, H. M.	11, 60
Yuan, D.	86
Yurchenko, O. P.	24
Zaplachinski, S.	24
Zhang, M.	24
Zhang, Y.	79
Zhang, Z.	37
Zhao, Y. Y.	67,87,88,89
Zope, D.	17
Zou, J.	25,94