2011 Annual Meeting Abstracts

Protein and Co-Products

MONDAY

MORNING

PCP 1: Co-Product Utilization from Biofuels

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

The Feed Opportunities from the Biofuels Industries: A Canadian Research Network. C.R. Christensen[1], J. McKinnon[1], T. McAllister[2], R. Ziljstra[3], A. Van Kessel[1], D. Anweiller[4], T. Fonstad[1], J. Hobbs[1], S. Smyth[1], [1]University of Saskatchewan, Saskatoon, SK, Canada, [2]Agriculture and Agrifood Canada, Lethbridge, AB, Canada, [3]University of Alberta, Edmonton, AB, Canada, [4]Saskatchewan Research Council, Saskatoon, SK, Canada

The Feed Opportunities from the BioFuels Industries (FOBI) Network is a multidisciplinary research network. The vision of FOBI is to stimulate the sustainable growth of the bio-ethanol and livestock sectors, leading to a stimulation of economic activities in rural Canada. The nutritionists within the FOBI network investigate feed constituents from wheat DDGS and their functionality in multiple livestock species. The wheat breeding group of FOBI focuses on identifying opportunities to improve the input side with new wheat varieties specifically for bio-ethanol and co-product output. The evaluation of the existing energy and fermentation models, focusing on optimization of ethanol processes, will enhance the understanding of the system of ethanol production, leading to enhancements in that system. FOBI is also assessing the impact of ethanol industry on economics of livestock industry and governance implications leading to the development of new markets and policies. Active collaboration with the ethanol manufacturers, related commercial entities and feedlots ensure that Ã¢â?¬Å?market-pullÃ¢â?¬Â • rather than Ã¢â?¬Å?technology-pushÃ¢â?¬Â • drives the FOBI Network.

Use of Enzymes to Improve Germ and Fiber Quality from Corn Dry Grind Fractionation Processes. V. Singh, E. Khullar, B.C. Vidal, K.D. Rausch, M.E. Tumbleson, University of Illinois at Urbana-Champaign, Urbana, IL, USA

To improve fractionation efficiency in modified dry grind corn processes, we evaluated the effectiveness of protease and phytase treatments to improve recovery and quality of coproduct (germ and fiber). In the first study, three schemes of protease treatment were conducted in three fractionation processes (E-Mill, dry RS and dry conv). At the end of fermentation, endosperm fiber was recovered and residual starch content was measured. Using protease treatment, residual starch in the endosperm fiber was reduced by 1.9% w/w (22% relative reduction) in dry conv and 1.7% w/w (8% relative reduction) in dry RS, while no reduction was observed in E-Mill. In the second study, effects of phytase addition on germ and pericarp fiber recovery were evaluated for the E-Mill process. Germ and pericarp fiber yields were compared for the E-Mill process with

and without phytase incubation. Phytase incubation had no effect on germ and pericarp fiber yields; however, germ oil contents were higher from the E-Mill process with phytase incubation (40.9%) than without phytase incubation (39.1%). Phytase treatment resulted in lower residual starch contents in germ and pericarp fiber (12.2 and 19.9%, respectively) compared to germ and pericarp fiber without phytase addition (18.1 and 27.4%, respectively).

Variation in Distillers Grains Quality and Investigation into Its Underlying Causes. Keshun Liu, USDA, ARS, Aberdeen, ID, USA

A major process for making ethanol from grains is the dry-grind method. The major co-product of the process is distillers dried grains with solubles (DDGS), which are widely used as a feed for animals and fish. Income from marketing of DDGS is important to the economic viability of the dry grind industry. Factors that affect quality or marketability of DDGS can impact its market value and end uses. It is well known to ethanol and feed industries and the scientific community that there is a great variation in physical properties and chemical composition among DDGS sources, even among batches of the same processing plant. This variation itself affects the marketability and end use of DDGS. For example, variation in protein content of DDGS can cause faulty formulation of feeds and thus affect animal productivity. This presentation discusses various possible causes for variation in DDGS quality based on limited studies conducted at the author's laboratory and elsewhere. These causes include effects of raw material, processing methods, treatments before, during and after the process, fermentation yeast, and analytical methodology. Such information can help us develop strategies to control quality variation, maximize balance of nutrients, and thus improve value-added utilization of DDGS.

Oxidative Stability of Distillers Grain Oils. J.K. Winkler-Moser, USDA, ARS, NCAUR, Peoria, IL, USA

The oxidative stability of any oil is dependent on intrinsic properties, such as fatty acid composition and content and composition of antioxidants, as well as on extrinsic factors, such as processing and storage. The oil in distillers grains is subject to extreme temperatures during processing, but several studies have shown that the fatty acid composition and antioxidant components in distillers grains oil are not greatly impacted during the dry grind ethanol process. However, free fatty acids and lipid oxidation products may accumulate in distillers grains, which can impact oil oxidative stability. The fatty acid composition, Acid Value, and the content and composition of tocopherols, tocotrienols, carotenoids, phytosterols, and steryl ferulates were determined in corn germ oil and four post-fermentation corn oils from the ethanol dry grind process. The oxidative stability index at 110ºC was determined for the five oils, and four oils were compared for their stability during storage at 40ºC as determined by peroxide value and hexanal content. Our results indicate that post-fermentation corn oils have higher content of valuable functional lipids than corn germ oil. Some of these functional lipids have antioxidant activity which increases the oxidative stability of the post-fermentation oils.

Ground Corn Processing to Food and Ethanol. Temur Yunusov[1], Rich Barton[1], Jon Hall[2], [1]Nutr-e Food Innovation Iowa, Osage, IA, USA, [2]Bio-NRG, Mt. Pleasant, IA, USA

Ground corn is a basic raw material for an ethanol production in USA. DDG'S is a by-product of

the process including some undesirable toxic substances of fermentation process side reactions. The approach including corn flour fractionation to final glucose solutions acceptable for the ethanol fermentaion process and food grade corn protein meal can potentially return million tons of a corn protein meal for a food application. The main issue of the approach is the quantitative separation of the corn ingredients from sugars acceptable for the existing ethanol industry. In the presentation the process stages including practically existing in ethanol industry process with some modifications to reach desirable goal will be considered. The final product test data including glucose solutions for ethanol and corn protein meal for human applications will be presented.

Novel Co-products from Renewable Diesel Technologies. D.C. Bressler, University of Alberta, Edmonton, AB, Canada

As the global economy shifts from nonrenewable energy sources to renewable ones, there is increasing need to develop integrated biorefining processes that produce co-products of higher value to increase the economic competitiveness of new technology platforms. Increasingly technology is enabling the rapid development and deployment of 2nd and 3rd generation biofuel technologies that provide options with superior compatibility to the traditional nonrenewable fuels. Over the past decade, research at the University of Alberta has focused on adapting technologies from the heavy oil upgrading sector for the conversion of plant oils, animal fats and other renewable triglyceride feedstocks to true hydrocarbon fuels and chemical replacements. Recent optimizations of these processes have identified co-product opportunities including short chain fatty acids and α-olefins. This presentation will focus on the development of a biofuel technology that captures the value of these higher value co-products and reports on process refinement activities including product recovery, conversion to continuous processing, and reaction optimization activities.

In situ Esterification Studies for Biodiesel Production from Various Feedstocks. Sevil Yücel, Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering,Department of Bioengineering, Turkey

Biodiesel is an alternative diesel fuel with low emission values produced from renewable resources such as rapeseed, sunflower, soybean and waste oils. Although it has many positive environmental effects it is not economically feasible fuel. Biodiesel is produced traditionally by transesterification reaction especially alkali catalyzed alcoholysis method. In the production of biodiesel from oilseeds, oil extraction, purification and transesterification steps are required. These steps constitute over 70% of the total biodiesel production when refined oil is used. Performing all stages in one step as in-situ esterification method can significantly decrease the production cost. Especially extraction and esterificiation proceed in single step and alcohol acts both extraction solvent for oil components and esterification reagent. In another words, this method eliminates using hexane solvent and subsequent evaporation. The another advantages of this process over the conventional process are evaluation of remained cakes as animal protein feed source and reducing toxic gossypol in cottonseed meal. This paper will review applications of situ esterification to oil seeds, (soy bean, sunflower, etc.) high acidity oil seeds (rice bran, Jatropha curcas L.) and other feedstocks (distillers dried grains with solubles-DDGS, meat and bone meal-MBM).

Cellulose Conversion Technologies for Utilization of Fiber-rich Corn Milling Co-products. Y.M. Kim[1,2], R. Hendrickson[1,2], E. Ximenes[1,2], N.S. Mosier[1,2], M.R. Ladisch[1,2], [1]Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN, USA, [2]Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN, USA

Faced with an escalating demand for fuel ethanol, researchers and ethanol industries are searching for ways to enhance ethanol yield by incorporating cellulosic ethanol technologies in corn biorefineries. Currently, bioethanol in the US is exclusively produced from corn starch via dry and wet milling processes, leaving the fiber fraction of corn kernels unutilized. Efforts have been directed to utilize and process these fibrous co-products, which are rich in polymeric sugars, such as cellulose, xylan and residual starch, into additional fermentable sugars and ethanol, while preserving their values as an animal feed. A hybrid corn-and-cellulosic ethanol production scheme would provide an opportunity to increase overall ethanol productivity of the current corn biorefineries with addition of pretreatment technology in the process. Research has shown that corn fiber and distiller's grains are easily hydrolyzed by cellulase enzymes and fermented by yeasts to produce ethanol. We report enzymatic digestibility and fermentability of pretreated distillers grains, effect of cellulase inhibitors, as well as compositional changes in the resulting co-products. Technological/economical barriers and process considerations in the modified corn biorefineries are also discussed.

Integration of Ethanol and Value-added Co-products in a Lignocellulose Biorefinery. Nhuan P. Nghiem, Eastern Regional Research Center, USDA ARS, Wyndmoor, PA, USA

Many attempts have been made over the last several years to develop technology for production of ethanol as a liquid fuel from lignocellulosic biomass feedstocks. Microbial strains have been isolated or developed for production of ethanol from both biomass-derived C5 and C6 sugars. Microbial conversion of C5 sugars to ethanol normally has low efficiencies. In addition, production of ethanol, which is a commodity chemical with small profit margin, as the only main product in a commercial plant with high capital costs results in a highly unfavorable overall process economics. Thus, we proposed a different strategy where only glucose is used for ethanol production and the C5 sugars are used for production of higher value-added co-products. In the first step biomass is pretreated by soaking in aqueous ammonia. The pretreated biomass is hydrolyzed first with commercial xylanases to generate xylose-rich streams, which then are used for production of the co-products. The residual solids are hydrolyzed with commercial cellulases to generate glucose-rich streams, which then are used for ethanol production. Fermentation of glucose is performed with the yeast Saccharomyces cerevisiae, which is the most efficient ethanol-producing organism widely used in the industry. The results of biomass pretreatment, fractionation, and fermentation will be discussed.

Triticale Distillers Grain Protein Extraction: A Possible Protein Source for Industrial Application. Nandika Bandara, Lingyun Chen, Jianping Wu, Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

Triticale wet distiller′s grains (WDG) and dried distiller′s grains with solubles (DDGS) were used to study the suitability of materials for protein extraction with the aim of use triticale

protein for industrial applications. Protein extractions were done using 0.05 N NaOH, 60% ethanol, Alkaline ethanol solution, glacial acetic acid with reducing conditions and Protex 6L enzyme to compare the extraction yield and protein content of extracts. Data were analyzed using one way ANOVA, followed by Duncan′s multiple range tests. Alkaline ethanol extraction and glacial acetic acid extraction were able to extract significant amount of proteins (~23–24% extraction yield) with comparatively higher protein content (~61–65%) than other extraction methods. Enzyme aided extraction was became the most successful method in extracting proteins where Protex 6L enzyme yielded 75–82% extraction yield with 43 to 57% protein contents depending on the type of raw material. Type of raw material has a significant effect on extraction yield with all extraction methods, but effect on protein content is varied depending on the extraction method. Protein fractionation study shows the differences in protein fractions comparatively to unfermented triticale grains, which is a result of excessive protein denaturation during bioethanol production.

Utilization of Co-products from Algae Biofuels. R.C. Green, POS Bio-Sciences, Saskatoon, SK, Canada

Algae offers considerable promise to constitute a significant portion of the renewable biofuels. In particular, algal lipids can effectively be extracted and converted to biodiesel. The utilization of co-products produced from the remaining residual biomass however, is critical in determining the commercial viability of algae production. Co-product energy can be obtained from the conversion of algae residual biomass to energy by combustion, pyrolysis or ethanolic fermentation. Valuable non-fuel co-products include the formulation of algae meal into animal and aquaculture feeds. In addition, algal protein concentrates and isolates have been reported to be of high nutritional quality. Lower volume, higher value co-products include nutraceuticals such as carotenoids and omega-3 rich oil fractions. There is also interest in the development of algae-based ingredients for cosmetics such as thickening agents and antioxidants. Although supported by significant research and development activity, the technology for the production of algae biofuels is still in the early stages of development and as such, algae-based co-products are still in the product development phases. The presentation will include a review of current and potential processes for algae biofuels and co-products.

Beyond Bieoethanol: Higher-value Chemicals from Residual Biomass. P. Champagne, A. Boyd, L. Zhang, V. Yates, P. Jessop, Queen's University, Kingston, ON, Canada

The recovery of bio-based products has important societal, economic and environmental implications. Scientific and technical progress in this field is dependent on the discovery of innovative routes in the extraction and synthesis of bio-based products, development and application of more environmentally benign processes; and recovery, reuse and valorization of process solvents, byproducts and waste streams.The thermochemical recovery bioenergy from "wet" biomass via direct liquefaction, a low-temperature, high-pressure process that takes place in the liquid phase, eliminates the need for energy intensive drying and densification required in other thermochemical processes. Using inexpensive catalysts, water contained in the "wet" biomass acts as a green solvent to produce bio-oil, bio-char and biogas. Algae are a promising biomass for the production of bioenergy with their high growth rate, higher photosynthetic efficiency than oil crops, high production capacity for bio-oil and potential for CO2

sequestration. The use of CO2 expandable and CO2 switchable solvents for the recovery of bio-oil from algae allows for effective product separation and solvent recovery under mild process conditions. The use of process wastewater streams and waste CO2 could enhance the benefits of this approach for CO2 sequestration and the mitigation of GHGs.

AFTERNOON

PCP 2: Alternative Plant Food Proteins and Co-Products

Chair(s): R. Aluko, University of Manitoba, Canada; and T. Yunusov, NFI Iowa, USA

Proteins of Chickpea and Lentils for Meat Industry Applications. Janitha Wanasundara[1,2], Thushan Sanjeewa[1,2], Kingsley Argyre[2], Phyllis Shand[2], [1]Agriculture and Agri-Food Canada, Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada

Chickpea, lentil and pea are pulses produced in Canada. Developing ingredients from pulses is vital to bring health beneficial properties to actual foods and also to increase use of pulses in regular foods. Pulses provide two valuable biopolymers, starch and protein, that are useful in food product formulations for various technological functions. This presentation will review the work we carried out on chickpea, lentil and pea protein ingredients for the use in meat products. Protein isolates were prepared by wet extraction at alkaline pH followed by acid precipitation. Functional performance of proteins was evaluated using established methods. The proteins were used in low-fat meat products formulations to evaluate the performance in key functional areas. Solubility of chickpea proteins was higher at pH 5 compared to lentil or pea which are scarcely soluble. At pH 7, lentil and chickpea showed complete solubility but pea protein did not. Pea and lentil protein isolates gave lower thermal denaturation temperatures than chickpea, and all were in the range of 82.2-93.2°C. Presence of NaCl in the medium provided a stabilization effect and increased denaturation temperature values for lentil and pea. These proteins can be incorporated into meat products successfully but functionalities and performance indicators showed some differences among these pulses.

Techno-functional Properties of Pulse Flours and Their Potential Use in Different Food Applications. J. Boye, Agriculture & Agri-Food Canada, Canada

Pulses contain between 18 - 32% proteins and are rich in lysine, leucine, aspartic acid, glutamic acid and arginine. These essential amino acids are often lacking in cereals such as wheat, rice, corn and buckwheat. Traditionally, legumes such as soybean which is also very rich in protein and contains high amounts of the essential amino acids lacking in cereals have been used in the diets of many cultures. Global demands for protein to feed the ever growing world population has created the need for alternative sources of proteins. Pulse (e.g., peas, chickpeas, lentils, beans) contain high amounts of protein which can be processed and used in a variety of food applications. In this study pulse flours were extracted using isoelectric precipitation and ultrafiltation. The initial protein content of the pulses (16.7-24.8%, w/w) was concentrated nearly 4 fold. UF process generated concentrates with slightly higher protein contents (69.1-88.6%, w/w) compared to the IEP process (63.9-81.7%, w/w). Yields for both processes on a protein

basis ranged between 50.3 to 69.1% (w/w). All concentrates exhibited good functional properties comparable to soy and whey proteins. Application areas include bakery, beverage and emulsion-type products.

Potential Utilization of Quinoa Seed Proteins and Hydrolysates as Functional Food Ingredients. Rotimi Aluko, University of Manitoba, Winnipeg, MB, Canada

Quinoa flour (from dehulled seeds) was extracted with aqueous NaOH followed by protein precipitation at pH 4.5 to give a concentrate with 65.52% protein content. The protein concentrate was hydrolyzed using alcalase and the resultant hydrolysate was fractionated by ultrafiltration through a 10 kDa molecular weight cut-off membrane. A second hydrolysate was also passed through 5 kDa membranes. Functional properties of the protein concentrate, protein hydrolysate and permeates from the 5 and 10 kDa membranes were compared at different pH values. Hydrolysis resulted in significantly increased (p

Relationship between Chain Conformation and Electrospinnability of Prolamin Proteins. Y. Wang, L. Chen, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Recent years have witnessed a rapid development of plant protein based biomaterials in non-food applications. Electrospinning is a broadly used technology for fiber formation and has seen a tremendous increase in research and commercial attention. However, there is a lack of enough efforts to involve prolamin proteins in the electrospinning industry. In this work, the electrospinnability of several prolamin proteins was demonstrated and compared for the first time. The relationship between chain conformations of pre-spun protein molecules and morphologies of post-spun fibers was investigated by CD spectroscopy, FTIR, TEM, laser light scattering and SEM. The results revealed that, although they exhibited similar amino acid components, their conformation and electrospinnability were quite different. The change of chain conformation from compact to extended structures led to the lowered and narrowed electrospinnable concentration and the transition from individual fibers to adherent ones. Moreover, large compact aggregates caused by hydrophobic interactions were found to have a negative effect on the formation of electrospun fibers. This fundamental work allows insight into inherent features that affect the manufacture of electrospun fibers from prolamin proteins, which are also useful for fabricating other prolamin protein based biomaterials.

Are Gluten "Free" Grains Such as Soy, Rice, Millett, etc. actually Gluten Free from Rye, Barley or Wheat Cultivars? Thomas Grace, Bia Diagnostics, Burlington, VT 05401, USA

Many flours and grains that are assumed to be naturally "gluten-free" (i.e. do not naturally contain the toxic prolamins that the Celiac Sprue community are sensitive to), may contain gluten through cross contamination via the field, transportation, storage or processing. How common is contamination in ?gluten-free? grains and what is the threshold level for Gluten Free labeling? In our study we tested twenty-two random inherently gluten-free grains, seeds, and flours not labeled ?gluten-free? purchased via the internet or local stores in June 2009 and sent unopened to Bia Diagnostics which specializes in gluten analysis. All samples were homogenized and tested in duplicate using the Ridascreen Gliadin sandwich R5 enzyme-linked

immunosorbent assay with cocktail extraction. Thirteen of 22 (59%) samples contained less than the limit of quantification of 5 parts per million (ppm) for gluten. Nine of 22 (41%) samples contained more than the limit of quantification, with mean gluten levels ranging from 8.5 to 2,925.0 ppm. Seven of 22 samples (32%) contained mean gluten levels >20 ppm and would not be considered gluten-free under the proposed FDA ruling for gluten-free labeling.

TUESDAY

AFTERNOON

PCP 3: Health Aspects of Food Proteins and Peptides

Chair(s): H. Kumagai, Nihon University, Japan; and H. Ibrahim, Kagoshima University, Japan

Casein Hydrolysates: Potential Bioactive Effects in Cultured Human Cells. N. O'Brien[1], M. Phelan[1], A. Aherne[1], D. O'Sullivan[2], R. Fitzgerald[2], [1]University College Cork, Cork, Ireland, [2]University of Limerick, Limerick, Ireland

Casein-derived bioactive peptides are encrypted within the primary structures of intact caseins and may be released during enzymatic hydrolysis, microbial fermentation and gastrointestinal digestion. Using cell culture systems, we have assessed the antioxidant, immunomodulatory and cytomodulatory activities of eight specific casein hydrolysates generated using different commercially available food-grade enzyme preparations from mammalian, bacterial and plant sources. The casein hydrolysates exerted varying effects on the viability and growth of Jurkat T cells, with IC50 values ranging from 19.5 to 66.8% (v/v). Treatment with the hydrolysates did not affect membrane integrity or superoxide dismutase (SOD) activity. Certain hydrolysates significantly affected both cellular catalase activity and glutathione content. Neither genotoxic nor genoprotective effects were exerted by the casein hydrolysates. Interestingly, a number of the casein hydrolysates significantly increased Concanavalin A (ConA) -stimulated IL-2 levels but had no effect on Con-A induced IL-10 production in the Jurkat cells. These findings suggest that casein-derived peptides may exert specific antioxidant and immunomodulatory effects on cells in culture.

Adding Value to Whey-How to Create Novel Bio-Functions in Whey Proteins and Peptides. Andre Brodkorb, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

B/HAMLET (Bovine/Human alpha-lactalbumin Made LEthal to Tumour cells) a complex of the whey protein alpha-lactalbumin and oleic acid with known tumouricidal and anti-microbial properties forms the basis of our molecular approach to a novel bio-functional application. Results showed the importance of the bound fatty acid to its biological function. The stoichiometry determined by FTIR/NMR can vary depending on preparations. In this paper we present a recently developed, non-chromatographic method to control both structure and stoichiometry of BAMLET, therefore facilitating the possible scale-up of this highly potent compound. The bioactivity of proteins and peptides is a cornerstone of Food for Health Ireland (FHI), partnership between Irish food industries and research organisations. The research focuses on the deconstruction of milk through fermentation and hydrolysis followed by subsequent bio-

screening and pre-commercial scale-up. Production of bio-active peptides with defined functional characteristics is largely dependent on processing parameters. The work presented here focused on the molecular optimisation of enzymatic hydrolysis through pre-treatment of the substrate. HPLC, SDS-PAGE, microscopy and FTIR provided a better understanding on a molecular and micro-structural level of the changes during protein aggregation and subsequent hydrolysis.

Ovotransferrin and its Peptides Confer in Vivo Resistance to Oxidative Stress. Hisham Ibrahim, Kagoshima University, Faculty of Agriculture, Kagoshima, Japan

Ovotransferrin (OTf) is an iron-binding protein in egg albumen with antibacterial activity and known as an acute phase protein in chicken serum, the level of which increase in inflammation and infections. We recently demonstrated that OTf undergoes self-cleavage under certain redox state, producing distinct peptides with SOD-like anti-oxidant action and anti-cancer activity to colon and breast cancer cells. It is assumed that OTf may be responsible for restoring homeostasis through modulation of processes related to oxidative stress or redox signaling and possibly help to resolve oxidative inflammation. However, the molecular basis of its action remained an enigma. For therapeutic and nutraceutical applications, it remains to be explored whether the same rules apply to the redox regulatory function by OTf in vivo. This study demonstrates, for the first time, that expression of OTf in eukaryotic cells confers resistance towards oxidative stress and the action correlated to the generation of OTf peptides within the reductive milieu of the cytoplasm. The results explores that peptides of the self-cleaved OTf represent novel therapeutic peptides with powerful antioxidant action in vivo that offer tremendous opportunities for their potential in the treatment of infection-induced oxidative inflammation.

Anti-fatigue Effect of Egg White Hydrolysate in Human Volunteers Mountain Climbing Test. Hajime Hatta[1], Namiko Suga[2], Mujo Kim[2], Seiichi Nakai[1], [1]Kyoto Women's University, Kyoto, Japan, [2]Pharmafoods International, Kyoto, Japan

Egg white, a low-fat and inexpensive source of protein, is considered as a suitable protein material for sport nutrition products. However, heat gelling and allergenic properties of egg white protein (EWP) have restricted its variety of uses in food. In this study, we performed hydrolysation of EWP by protease to eliminate heat gelling and allergenic properties of EWP and determined the anti-fatigue effect of oral administration of EWP hydrolysate (EWPh) (M.W.

Cancer Anti-proliferative Activities of a Pentapeptide Derived from Rice Bran. Arvind Kannan, Navam Hettiarachchy, University of Arkansas, Fayettevile, AR, USA

Food proteins and biopeptides promote functional activity against diseases in addition to their established nutritional value as sources of protein. The purpose of our study was to isolate and characterize peptides derived from rice bran for anti-proliferative properties. Gastrointestinal juices resistant peptide fractions were prepared from heat stabilized de-fatted rice bran from which the anti-proliferative

Suppression of Postprandial Hyperglycemia by Cereal Protein. Hitomi Kumagai, Nihon

University, Fujisawa-shi, Japan

Diabetes mellitus is a group of disorders characterized by hyperglycemia. Approximately 90 percent of all cases of diabetes are type 2 that shows a symptom of insulin resistance and/or deficiency. In order to prevent type 2 diabetes, it is important to control the blood glucose level in our daily life by an appropriate food intake. Inhibition of α-amylase activity and retardation of starch hydrolysis is one of the effective ways to suppress the blood glucose level, and wheat α-amylase inhibitor is the well-known protein that possesses the strong inhibitory activity against α-amylase. However, the activity of protein in other cereals has not fully examined yet. Therefore in this study, α-amylase inhibitors were purified from buckwheat and rice, and their suppressive effect on postprandial hyperglycemia was examined. Buckwheat albumin inhibited porcine pancreas α-amylase activity, but did not inhibit human salivary α-amylase activity. Although it was readily hydrolyzed by digestive enzymes, it suppressed postprandial hyperglycemia after carbohydrate loading. On the other hand, rice albumin did not inhibit both porcine pancreas and human salivary α-amylase activities. However, it showed strong heat resistance as well as digestive tolerance. It suppressed postprandial hyperglycemia not only after carbohydrate loading but also after glucose loading.

In vitro Bile Acid Binding Properties of Lentil Proteins and Hydrolysates. J. Boye, C. Barbana, Agriculture and Agri-Food Canada, Canada

Lentil is a leguminous crop with excellent nutritional properties. Recent research has shown that lentil proteins may have in vivo ACE inhibitory properties which might make them beneficial for reducing cardiovascular disease (CVD) risk. Another mechanism proposed to decrease CVD risk is cholesterol reduction. Disruption of the reabsorption of bile acids through binding to bile acid sequestrants leads to further degradation of cholesterol in the liver, reducing its level in blood. In this study, the binding capacity of bile salts by lentil flours and lentil protein concentrates and hydrolysates were evaluated. Sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate and sodium chenodeoxycholate were tested individually, and their binding interactions with the lentil products were analyzed and compared to cholestyramine. All tested samples bound the bile salts investigated, and the amount of bile salts bound (>70%) was sometimes greater than that bound by cholestyramine. In vitro digestion of the lentil proteins by pepsin/trypsin/α-chymotrypsin, alcalase/flavourzyme and papain significantly reduced the bile salt binding capacity compared to the undigested samples in most cases. The ability to bind bile acids could open new opportunities for the use of lentil products in the formulation of functional foods.

Influence of Amino Acid Supplementation on Dietary β-conglycinin-dependent Reduction of Food Consumption and Modulation of Lipid Metabolism in Rats. K. Koba[1], D. Oikawa[2], S. Tamaru[1], K. Tanaka[1], M. Sugano[3], [1]University of Nagasaki, Siebold, Nagayo, Nagasaki, Japan, [2]Nagasaki University, Nagasaki, Japan, [3]Professor Emeritus, Kyushu University, Fukuoka, Japan

Beta-conglycinin (CON) is one of the major protein components of soy protein (SOY), and is suggested to be responsible for reducing serum and liver triglyceride concentrations. We previously observed that feeding of CON as compared with casein (CAS) and SOY lowered the

body weight gain in rats, due to a significant decrease of food consumption. Compared with CAS, CON contained less essential amino acids, such as Thr, Val, Met, Tyr and Trp. Then, we examined how the supplementation of these amino acids to CON affects food consumption and lipid metabolism in rats. Rats were fed the diets containing 20% protein; either CAS, SOY, CON or CON supplemented with the five amino acids to make the levels equal to CAS. After 4-week feeding period, dietary CON supplemented with amino acids completely ameliorated the CON-dependent decrease of food consumption. Also, CON supplemented with amino acids exerted a decrease of serum and liver triglyceride concentrations at least to the comparable level to SOY did. The results indicated that essential amino acid profile is at least responsible for a CON-dependent reduction of food consumption. The results in the present study provide basic information to elucidate physiological functions of CON.

Impact of Extracellular Matrix Protein Hydrolysates on Human Health. Kenji Sato, Kyoto Prefectural University, Kyoto, Japan

Collagen and elastin are main constituents of extracellular proteins. These proteins are contained in byproducts from slaughterhouse and food industry. Recently, enzymatic hydrolysates of these poteins have been prepared in an industrial scale and used for functional food ingredient. There are episodes suggesting that ingestion of these products improve skin and joint conditions. However, little were known for mechanisms underlying the beneficial effects. We hypothesized occurrence of food-derived collagen and elastin peptides in human circulation system, which could be responsible for the beneficial effects. The objective of the present study was to indentify the food-derived collagen and elastin peptides in human blood and examine their potential biological activity by in vitro assay. Pro-Hyp, Hyp-Gly, Ala-Hyp and other minor peptides were identified as food-derived collagen peptides in human peripheral blood. For food-derived elastion peptide, Pro-Gly was identified. The food-derived collagen and elastin peptides enhance growth of fibloblast on collagen gel and vein endothelial cell, respectively. These data suggest these dipeptides might exert beneficial effects on skin and joint via enhancement of healing process of damaged connective tissues and improvement of blood circulation.

WEDNESDAY

MORNING

PCP 4: Functional Properties of Proteins and Co-Products

Chair(s): S. Jung, Iowa State University, USA; and P. Kerr, Solae Co., USA

****Cancelled** Physiological Activities of Amaranth Proteins.** M.C. Añón, CIDCA, UNLP ? CONICET, CCT La Plata, Argentina.

Effects of Seed Preparation and Oil Pressing on Milkweed (*Asclepias spp.*) Protein Functional Properties. M.P. Hojilla-Evangelista, R.L. Evangelista, USDA, ARS, NCAUR, Peoria, IL, USA

The effects of seed cooking and oil processing conditions on functional properties of milkweed seed proteins were determined to identify potential value-added uses for the meal. Milkweed seeds were flaked and then cooked in the seed conditioner at 82°C for 30, 60 or 90 min. Oil was extracted by screw-pressing. Functional properties (solubility, foaming, emulsification, water-holding capacity) of extractable proteins in cooked flakes, press cakes, and unprocessed ground, defatted milkweed seeds were determined and compared. Milkweed seed protein was least soluble at pH 4 and solubility increased steadily with pH until the maximum (60%) was reached at pH 10. Seed proteins also had notable emulsifying and foaming capacities. Cooked seeds and press cakes showed protein solubility profiles that were similar to that of the unprocessed, defatted seed and also had improved emulsifying, foaming, and water-holding capacities, which indicated that the heat applied during these steps had no deleterious effects on protein functionality. These results showed that protein in milkweed seed and its press cake from oil processing has functional properties that would be useful in various industrial applications.

Rapeseed Proteins Extraction and Enzymatic Hydrolysis: Assessment of Products Functionalities. R. Kapel, C. Nioi, C. Harscoat-Schiavo, F. Fournier, I. Chevalot, I. Marc, LRGP, UPR CNRS 3349, Nancy, France

Rapeseed is an oil-rich-crop originally cultivated for the production of food oil intended to human consumption. The industrial oil extraction process from rapeseed leads to the production of a protein-rich co-product. The rapeseed culture has been greatly increasing for around a decade, as rapeseed oil started to be used as a substrate for bio-fuel production. Furthermore, it has become important to rationalize the use of each co-product from agricultural product transformation. In this context, a better valorization of the rapeseed meal is required, that can be achieved thanks to their proteins content, either directly or after enzymatic hydrolysis. This study presents an integrated research activity aimed at evaluating the rapeseed proteins potential of use for food safety and animal cells culture as well. Results will be presented concerning (i) processes for global meals proteins extraction and targeted meal proteins selective extraction, up to pilot scales, taking into account qualitative and quantitative criteria, (ii) functional properties and anti-microbial activity of extracted proteins, (iii) proteins transformation by enzymatic hydrolysates and (iv) hydrolysates promoting effect on animal cell growth.

Improving the Meat Functionality of Soy Protein Concentrate Through Fiber and Protein Modification. Der-Chyan Hwang, Bill Monagle, Tom Mertle, Ted Wong, Phil Kerr, Solae, LLC, St. Louis, MO, USA

Since there is about 25 to 30 % fiber present in soy protein concentrate (SPC), manipulating the composition and swelling characteristics of fiber portion in SPC could be a potential approach to improve the functionality of SPC in emulsified meat system (EMS). This study has employed various analytical techniques, such as Rapid Visco Analyzer (RVA), Particle Size Analyzer and Size Exclusion Chromatography to identify the potential effect of process conditions on viscoelastic properties of fiber in SPC. The combination of alkaline and heat treatment was found to be able to efficiently modify the swelling characteristics of SPC. The modified SPC showed greater emulsion strength and improved performance in EMS application. The formation of soluble protein aggregates and the reduction of insoluble dietary fiber (28-55%) by the

alkaline/heat treatment seemed to be responsible for the increase in emulsified meat functionality of SPC.

Soy Protein Functionality Improvement by Hydrolysis Using Serine Proteases. Naina Shah, Der-Chyan Hwang, Ted M. Wong, Zebin Wang, Barry Tulk, Jason F. Lombardi, Phil Kerr , Solae Co., USA

Use of soy proteins is a sustainable solution to the increasing demand for protein for the world?s increasing population. However utilization of soy proteins in various applications is limited by the functional characteristics of soy proteins, these include solubility over a range of application pHs, high viscosity, color, flavor etc. This work describes hydrolysates generated using 2 different serine proteases to generate hydrolysates with improved functionality. A serine protease (SP1) was utilized to generate hydrolysates with degrees of hydrolysis between 0-10% which yielded hydrolysates with increased solubility in the acidic pH range suitable for sports beverage applications. Another serine protease (SP2) was utilized to generate hydrolysates that had improved whiteness, imparted unique rheological properties to the hydrolysates as well as allowed to obtain the 47kDa core region from beta conglycinin which is thought to be responsible for cholesterol lowering.

A New Method to Determine the Carbohydrate Profile in Soy Fiber and Oil Seeds. T. Tran, B. Pierce, W. Perez, Solae LLC, St. Louis, MO, USA

Soy fiber and oil seeds are a rich source of complex carbohydrates. However, much research is still required to provide a complete characterization of these carbohydrates at the molecular level. In order to address these needs, a new approach to effectively and reproducibly determine the sugar profile of soy fiber was developed. The novel sugar profile analysis described in this paper comprised an improved acid hydrolysis of the soy fiber with sulfuric acid at high temperature and high pressure and a subsequent quantitative identification of the resulting sugars using HPAE-PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection). The modified acid hydrolysis assay included a pretreatment of Soy Fiber with 12M sulfuric acid at 30°C for 1 hour, and after dilution, a subsequent autoclave treatment at 121°C and 18 psi for 1 hour. The HPAE-PAD sugar analysis showed excellent reproducibility and was able to differentiate and quantify several mono- and oligosaccharides, including those of isomeric structures like galactose and glucose. As demonstrated, this combination provides a more complete picture of the hydrolyzed carbohydrate profile of soy fiber. This analytical method can be applied not only to other soy-based materials, but also to other carbohydrate-containing oil seeds and cereal grains.

Bioactive Peptide in Soybean induces Apoptosis in Human Metastatic Colon Cancer Cells. E. Gonzalez de Mejia, V. Dia, University of Illinois, Urbana, IL, USA

We evaluated the potential of the soybean peptide lunasin to induce apoptosis in human colon cancer cells and their oxaliplatin-resistant (OxR) variants. Various human colon cancer cell lines, which underwent metastasis following a mouse model, were evaluated using cell flow cytometry and fluorescence microscopy. Lunasin cytotoxicity to different colon cancer cells correlated with the expression of α5β1 integrin; most potent to KM12L4 cells (IC_{50} = 13 μM), arrested phase

G2/M with concomitant increase in the expression of cyclin dependent kinase inhibitors p21 and p27. Lunasin (5- 25 μM) activated apoptosis by changes in the expressions of Bcl-2, Bax, nCLU, cytochrome c and caspase-3 in KM12L4 and KM12L4-OxR. It also increased the activity of initiator caspase-9 leading to activation of caspase-3, and also modified the expression of human extracellular matrix and adhesion genes; down-regulated integrin α5, SELE, MMP10, integrin β2 and COL6A1 by 5-, 6-, 7-, 8- and 10-fold, respectively while up-regulating COL12A1 by 11-fold. The results suggest that lunasin can be used in cases where resistance to chemotherapy develops.

Hydrolyzed Soy Protein Contains Bioactive Peptides that Release Cholecystokinin from Enteroendocrine Cells. B. Tulk, N. McGraw, J. Li, N. Napawan, D. Butteiger, J. Lombardi, Z. Wang, K. Moore, E. Krul, Solae, LLC, St. Louis, MO, USA

The inclusion level of protein in many food applications has grown in recent years due in large part to the drive by consumers to either gain or maintain lean body mass. Soy is a source of nutritionally complete plant protein that could potentially be used in several of these applications, but often it is a challenge to incorporate intact soy protein due to its physicochemical properties (e.g., high viscosity, low solubility in acidic environments). Partial proteolytic digestion of soy proteins solves many of these issues. We have also noted that the resultant peptides have increased bioactivity through our in-house cell-based screening assays. In this paper, we present examples of partially hydrolyzed soy proteins that exhibit an enhanced ability to release the hormone cholecystokinin (CCK) from enteroendocrine cells compared to intact protein or other hydrolysates. CCK is believed to be at least partially responsible for the feeling of satiety associated with protein consumption. We have generated hydrolysates that elicit an enhanced CCK release response using several different enzymes. The release varies with the specific enzyme used as well as the degree of hydrolysis, strongly suggesting there are specific components of the hydrolysates that are responsible for CCK release.

Extraction and Fractionation of Protein Derived Bioactive Peptides by using Various Chromatography Techniques. Mahsa Naghshineh[1], Hasanah Mohd Ghazali[1], Hamed Mirhosseini[2], Sadra Tabassi[3], [1]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, [2]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, [3]Graduate School of Management, Universiti Putra Malaysia (UPM), Malaysia

The value of proteins as an essential source of amino acids is well demonstrated. It has been recognized that dietary proteins exert different functionalities in vivo by means of biologically active peptides. Bioactive peptides (BAPs) are specific protein fragments with a positive impact on body functions. Novel technologies such as reversed-phase liquid chromatography (RPLC) and ion-exchange liquid chromatography (IELC) are considered as powerful methods for peptide separation in both the analytical and preparative mode. Ion exchange chromatography (IEC), size-exclusion chromatography (SEC) and solid-phase extraction (SPE) were employed as fast becoming indispensable tools in various research areas and may consider as chromatographic process to isolate peptides. However, an efficient method for the separation of bioactive peptides is required. For instance, RPLC is an inappropriate technique for separation of highly cationic

peptides and in this case IELC is the convenient method. The present review mainly contemplate on the type and the classification of chromatography techniques applied for fractionation protein derived bioactive peptides and screen the most suitable chromatographic process for isolation of bioactive peptides.

AFTERNOON

PCP 5: General Protein and Co-Products

Chair(s): N. Deak, Solae Co., USA; and P. Qi, USDA, ARS, ERRC, USA

****Cancelled** The Effects of Proteins in Simple Starch Matrices.** C. Onwulata, USDA, ARS, ERRC, USA

Application of Enzyme-assisted Aqueous Oil Extraction to Peanut. Yingyao Wang, Aike Li, Xia Luan, Chuanling Du, Rong Ma, Academy of State Administration of Grain, Bejing, China

Enzyme-assisted aqueous oil extraction is a safe and efficient oil extraction process that may also result in edible protein hydrolysates. In this study, blanched peanut splits were ground and dispersed in 5 parts (wt/vol) of water. The extraction was done at 60°C, pH 8.50 for 1h and then the liquid extracted was submitted to an enzymatic reaction step by using alcalase2.4L to obtain free oil and protein hydrolysates. Under the condition of pH8.50, 60°C, an enzyme level of 1.5% and 5h incubation, an oil yield of 73.2% and hydrolysates yield of 83.2% were achieved. Oil yield was improved to 91.5% after demulsification by freezing at -16°C and thawing at 35°C. The molecular weight of the most peptides in protein hydrolysates was less than 8750Da. But the gel filtration of the protein adsorbed at the emulsion interface showed 3 main peaks with molecular weight of 727 kDa, 232 kDa and 108 kDa, respectively. The protein hydrolysates have good effects of scavenging the α,α-diphenyl-β-picrylhydrazyl(DPPH) free radical and inhibiting the angiotensin-I-convertine enzyme (ACE).

Development of Microalgae Food Ingredients. Leslie M. Norris, Solazyme, South San Francisco, CA, USA

Solazyme is a renewable oil and bioproducts company, harnessing the power of microalgae to renewably produce clean and scalable fuels, chemicals, and foods. Solazyme is now entering into a commercial phase, producing tens of thousands of gallons of algal oil that transform carbohydrate feedstocks into renewable triglyceride oils. Through Solazyme?s algal food ingredient platform, the company is able to create: ? Food ingredients that are rich in heart-healthy unsaturated fats and contain no trans-fats or cholesterol? Are shelf stable and composed of a variety of valuable fatty acid profiles? Food oils that are high in antioxidants and other valuable nutritional materials, including phospholipids, tocopherols, tocotrienols, sterols and carotenoids? Are not genetically modified in any formSolazyme has demonstrated that when used in full or partial replacement of full-fat lipids (eggs, butter, oil), significant reduction in

calories, saturated fat, and cholesterol can be achieved, while adding micronutrients and dietary fiber. The products also provide a number of functional benefits such as enhanced sensory qualities in low-fat formulations and improved moisture retention in challenging applications such as gluten-free recipes. Thus, Solazyme is addressing consumer demand for natural foods that have healthier nutritional profiles, while also providing ingredients that don?t sacrifice taste.

Hydrolyzable Tannins from Different Plant Species: Their Potential Uses in Agriculture and Biomedical Sciences. A. Romani[1], S. Miele[2], E. Bargiacchi[3], M. Campo[1], P. Buzzini[4], [1]Department of Pharmaceutical Sciences, University of Florence, Firenze, Italy, [2]Department of Agronomy and Agroecosystem Management, University of Pisa, Pisa, Italy, [3]Consortium INSTM, Firenze, Italy, [4]Department of Applied Biology - Microbiology, University of Perugia, Perugia, Italy

Tannins are distributed in several plant species, organs and tissues, where they play a role in inhibiting microbial decay, thus enhancing material durability. In the hydrolysable tannins (HTs), a carbohydrate molecule (usually D-glucose) is partially or totally esterified with phenolic groups, such as gallic or ellagic acids (gallotannins, GTs; or ellagitannins, ETs). HTs are hydrolyzed by weak acids or bases, and are more easily oxidized than condensed tannins (CTs). In the present work, several different water extracts, high in HTs, obtained from plants such as chestnut (*Castanea sativa* Mill.), myrtle (*Myrtus communis* L.) and pomegranate (*Punica granatum* L.), have been analysed and characterized by HPLC/DAD and HPLC/MS methods. Chestnut whole water extract (13% HTs) was investigated in agriculture as a natural soil acidifier, salinity control agent, phosphate solubilizer, iron complexing agent, and nemastat product. Aqueous extracts of the three plant species were also fractionated using both resins and membranes to recover purified molecules as bio-phenols for testing their antimicrobial activity. Novel HTs extracts exhibited antioxidant and antimicrobial activities, potentially prospecting they could support or even substitute molecules of current clinical use.

Structure-function Properties of Hemp Seed Globulins and Albumins. Rotimi Aluko, Anne Yvart, University of Manitoba, Winnipeg, MB, Canada

Two major protein fractions (globulin and albumin) of hemp seed were isolated and their physicochemical and functional properties investigated. For some of the investigated properties, the effects of pH and NaCl concentrations were also evaluated. Albumin had significantly higher (p

Identification and Characterization of Sphingosine Binding Protein. Zakir Hossain[1,2], Taro Masuda[3], Tsuyoshi Tsuduki[4], Tatsuya Sugawara[1], Takashi Hirata[1], [1]Kyoto University, Kyoto, Japan, [2]Bangladesh Agricultural University, Mymensingh, Bangladesh, [3]Osaka University, Osaka, Japan, [4]Institute for Protein Research, Osaka University, Osaka, Japan, [5]Tohoku University, Sendai, Japan

The Caco-2 cells were cultured up to 14-21 days for differentiation. A 40 Î¼M sphingosine with FBS free medium was added and incubated for 2 h. Sphingsine bases treated cells were lysed and sample was loaded to the DEAE column at a flow rate 1ml min-1. Fractions were analysed on fluorescence detector HPLC system. Sphingosine was identified comparing the peak of a

standard solution. Identified fraction was put into a 3-kDa cut-off centrifugal filter and centrifuged at 13000 rpm for 20 min until around 80% of the fluid had passed through the filter. SDS-PAGE was carried out using 15% acrylamide gels. Bands were excised from the gels. Protein pieces were washed sequentially with acetonitrile and ammonium bicarbonate. Proteins were reduced by treatment with 100 mM DTT for 30 min, and alkylated with 100 mM indoacetoamide. The digestion was accomplished with 25 Î¼g/ml trypsin at 37oC overnight. Tryptic peptides were analyzed by MALDI-TOF. The major peaks obtained by MALDI-TOF were selected to be further characterized by TOF/TOF analyses. Spectra were submitted for database searching in a generic MASCOT format. The identified sphingosine binding protein is PDIA3. These results suggest that absorption of bioactive sphingosine takes place in the human intestine through binding with PDIA3.

Characterization of Bovine Blood Proteins with Flocculation Activity. G.J. Piazza, A. Nuñez, R.A. Garcia, Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA, USA

Bovine blood is an excellent flocculating agent, faster acting and nearly as effective on a mass basis as polyacrylamide, the most widely utilized polymeric flocculant. To determine the molecular basis of flocculation activity, whole bovine blood (BB) and BB plasma were fractionated by size exclusion chromatography. The BB fraction with highest flocculation potential (FP) was subjected to preparative electrophoresis, and the tryptic peptides of the major protein component were examined by matrix assisted laser desorption/ionization with automated tandem time of flight measurement of selected ions (MALDI-TOF/TOF) mass spectrometry. Hemoglobin dimer (subunits α and β) was identified as the major protein flocculant; its high FP was confirmed by testing a commercial sample of hemoglobin. Three plasma proteins were also found to have flocculation activity, but bovine serum albumin was not a flocculant.

****Cancelled**** **Properties of Whey Protein Based Biocomposite.** S. Mukhopadhyay and C. Onwulata, USDA, ARS, ERRC, USA.

Protein and Co-Products Posters

Chair(s): J. Wu, University of Alberta, Canada

Rapeseeds: A Potent Feedstock for High-valued Biomolecules Production using Green Processes.
L. Leitner[1,2], R. Kapel[1], A.-L. Elfassy[2], I. Marc[1], L. Muniglia[2], [1]LRGP, UPR CNRS 3349, Nancy, France, [2]LiBio, Nancy, France

Rapeseeds are of a great interest because of their high content in several valuable components, (oil, proteins, polyphenols and carbohydrates). Nowadays, rapeseeds are processed at industrial scale for oil production (for human consumption and bio-fuels). The current industrial oil processes are based on polluting solvent extraction (by VOC emission). Aqueous and enzymatic oil extraction might be a promising way for a global rapeseed sustainable bioraffinery since it (i)

is far more eco-friendly and (ii) leaves an aqueous effluent rich in compounds, having high potentialities. In our study, an aqueous and enzymatic extraction method was developed to obtain free oil and a protein isolate from rapeseed seeds. In order to reduce the formation of emulsion during the hydrolysis of cell wall components, a pre-extraction of the proteins was investigated at a pH value of 10. This step allowed to increase the free oil yield from 11 % to 47 %. The aqueous phase resulting from the protein pre-extraction was then ultrafiltered to improve its protein purity. This step allowed to enhance the purity in nitrogen matter from 48% to 77%, and simultaneously to reduce the peptids/proteins (weight/weight) ratio from 0.16 to 0.04.

Emulsifying and Physicochemical Properties of Protein Isolates from Chickpea, Faba Bean, Lentil, Pea, and Soy.

Asli Can Karaca, Andrea Stone, Nicholas Low, Michael Nickerson, Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada

The emulsifying (emulsion capacity, EC; emulsion activity/stability indices, EAI-ESI and creaming stability, CS) and physicochemical (surface charge/hydrophobicity, protein solubility, interfacial tension, and droplet size) properties of chickpea (ChPI), faba bean (FbPI), lentil (LPI), and pea (PPI) protein isolates produced by isoelectric precipitation method were investigated relative to soy (SPI). All proteins carried a net negative charge at neutral pH. Surface hydrophobicity ranged from 55.2 to 84.8; PPI showing the highest value. Solubility was found to be lowest at 61.4% for PPI, then increased to ~90.2% for FbPI ≈ LPI, then again to ChPI, and followed by SPI (96.5%). EC ranged between ~478 to ~520 g oil per g protein; with SPI showing the highest capacity. The EAI values for PPI, SPI, FbPI and LPI were similar (42.9-44.5 m^2/g), whereas values for ChPI were significantly higher (47.9 m^2/g). ChPI, SPI and LPI showed higher ESI (~84.0 min) compared to FbPI (69.3) and PPI (12.5 min). All protein isolates formed emulsions with similar droplet size (~1.6 μm) and showed high CS (~98.1%). Findings suggest that chickpea and lentil proteins have the potential to serve as an alternative to soy protein, for stabilizing oil-in-water emulsions.

Novel Antioxidative Peptides from Cereal Protein.

Y. Xia, L. Chen, University of Alberta, Edmonton, Alberta, Canada

Antioxidative peptides or protein hydrolysates have high potential to be widely applied for food safety and human health. The objective of this research was to perform barley glutelin enzymatic hydrolysis to produce antioxidant peptides with a focus on protease type and hydrolysis time on subsequent peptide structure and antioxidant capacity. The hydrolysate molecular weight, hydrophobicity and secondary structure were characterized by ultrafiltration, reverse-phase HPLC, spectrofluorometer and Fourier transform infrared spectroscopy methods. Their antioxidant activities were evaluated through free radicals scavenging, metal chelating, and reducing power assays. The results revealed that the high molecular weight peptides (>10 kDa) possessed stronger capacity in DPPH free radical scavenging and reducing power while the low molecular weight hydrolysates (

Properties of Pea Protein Isolate with Pressure Treatment and Thermal Treatment.

Dongfang Chao[1], Stephanie Jung[2], Rotimi Aluko[1,3], [1]University of Manitoba, Winnipeg, MB, Canada, [2]Dept. of Food Science and Human Nutrition, Iowa State University, Ames, IA, USA,

[3]Richardson Centre for Functional Foods and Nutraceuticals, Winnipeg, MB, Canada

The influence of high pressure and thermal treatment on the functional properties on the pea protein isolate (PPI) was investigated in this study. No significant change was observed in the solubility of PPI after high pressure treatment. However, high pressure treatment could markedly improve the emulsifying property of PPI with smaller oil droplet, especially PPI with 200 MPa treatment at pH=3. On the other hand, emulsions formed by PPI under thermal treatment had larger oil droplet size than those formed by native PPI suggesting that thermal treatment has adverse effect on the emulsifying property of PPI. In addition, PPI with high pressure treatment also showed better foam properties than PPI. These results showed that PPI with pressure treatment could be applied to improve the quality of PPI stabilized food systems.

Qualitative Analysis of Milk Protein Hydrolyzing Enzymes from Various Sources.

Mahsa Naghshineh[1], Hasanah Mohd Ghazali[1], Hamed Mirhosseini[2], Sadra Tabassi[3], [1]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, [2]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, [3]Graduate School of Managment, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia

Dietary proteins such as milk proteins possess a wide range of nutritional and functional properties due to the presense of physiologically active peptides. The bioactive peptides may be liberated from the protein by enzymatic hydrolysis in the gastrointestinal tract, or before consumption caused by the activity of native milk enzymes or throughout food processing. Milk protein hydrolysates are mainly used as protein-based ingredients in food, beverage or as a main component for non food applications. Enzymatic hydrolysis has become extensively used biotechnological process to achieve proteins with improved functional properties. Specific properties of the protein hydrolysates are dependent on the degree of hydrolysis (DH) influenced by the specific activity of the protease, physical and chemical characteristics of the protein substrate, reaction conditions and type of protein hydrolyzing enzyme. Currently, protein hydrolyzing enzymes are commercially available from various plant, animal tissue and microbial sources. This review summarizes different milk protein-hydrolyzing enzymes from various sources (namely plant, microbial and animal) providing hydrolyzed milk proteins with diverse biological activities.

Pretreatment of Soybean Fiber by Soaking in Aqueous Ammonia Prior to Saccharification.

Bishnu Karki[1], Devin Maurer[1], Tae Hyun Kim[3,4], Stephanie Jung[1,2], [1]Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, USA, [2]Center for Crops Utilization Research, Iowa State University, Ames, IA, USA, [3]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, USA, [4]Department of Natural Resources, Ecology and Management, Iowa State University, Ames, IA, USA

The effectiveness of soaking in aqueous ammonia (SAA) as a pretreatment method for the conversion of fiber-rich insoluble soybean fractions to glucose via enzymatic hydrolysis was investigated. The fiber-rich insoluble fractions obtained from two-stage countercurrent enzyme-assisted aqueous extraction processing (EAEP) of full-fat soybean flakes (FFSF) and extruded FFSF were soaked in 15 wt% aqueous ammonia at a 1:10 solid-to-liquid ratio (SLR). The effect

of operating variables, i.e., soaking times (6, 12 and 24 h), soaking temperatures (60 and 80 °C) and enzyme loadings (15 and 60 FPU/g-glucan) on degree of enzymatic hydrolysis were studied. At the best SAA conditions, i.e., 80 °C for 12 h and an enzyme loading of 15 FPU/g-glucan, the glucose yield of insoluble fractions from FFSF was 57% after 48 h of hydrolysis, which was 5.2 times that of untreated insoluble fractions. This glucose yield was increased to 88% with extruded fiber fractions when pretreated conditions remained identical. Our results indicate that SAA is a simple and technically feasible pretreatment method for the conversion of fiber-rich insoluble fraction to fermentable monomers via enzymatic hydrolysis.

Soy Protein Isolate (SPI) and Milk Whey Protein Isolate (MWPI) Interfacial and Foaming Properties Study.

C. Abirached[1], A. Medrano[1], I. Vieitez[1], L. Panizzolo[1], P. Moyna[1], M. Añón[2], [1]Facultad de Química, Universidad de la República, Montevideo, Uruguay, [2]CIDCA, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

A comparative study on the foaming properties and viscoelastic behavior at the air-water interface of milk whey and soy protein isolates was made.Foams were obtained by the method of gas bubbling and the conductivity was recorded as a function of time. There were determined the initial rate of passage of liquid to the foam (Vo) and the maximum volume of fluid incorporated to the foam (VLEmax). The process of destabilization of foams formed was analyzed from the data which were fitted to a biphasic second-order kinetics characterized by the parameters kg and kd. kg is the rate constant of fluid drained by gravity and kg corresponds to the liquid drained by gas diffusion.The surface tension and the surface rheological properties of films adsorbed at the water/air interface were measured.The foam ability (Vo and VLEmax) and the stability (kg and kd) of the foams made with MWPI were better. As for surface tension measurements, there were found no significant differences ($\alpha \leq 0.05$). In contrast, there were significant differences in the dilational modulus, being higher with MWPI, implying greater resistance of the film formed and explaining the greater stability of foams.

Effect of High Temperature Fuel Ethanol Processing on the Functional Properties of Wheat Protein Co-products.

N. Avramenko, A.K. Stone, T. Haji, M.T. Nickerson, University of Saskatchewan, Saskatoon, SK, Canada

The effect of high temperature fuel ethanol processing on the functional properties of wheat proteins (i.e., gliadin and glutenin) were examined at various stages during the process (e.g., post-liquefaction (PL), post-distillation (PD – whole stillage) and post-drying (DDGS – distillers' dried grains with solubles)) relative to the wheat feedstock. The wheat feedstock and the PL co-product showed similar water hydration capacities (WHC) ranging between 80 and 110%, whereas WHC for the PD and DDGS co-products were significantly higher (~264-356%). A similar trend was found for the oil holding capacities (OHC) where both the feedstock and the PL co-product showed lower values (~120%) than both the PD (203%) and DDGS (171%) co-products. Heat treated wheat proteins (PL, PD and DDGS) also showed significantly reduced foaming and emulsifying properties relative to the wheat feedstock. Findings suggest that pre-processing the wheat feedstock for protein prior to entering ethanol fuel production is needed to preserve the quality and value of the protein co-products.

Kinetics of Enzyme Inhibition and Antihypertensive Effects of Hemp Seed (*Cannabis sativa* L.) Protein Hydrolysates.

A. Girgih[1,2], C. Udenigwe[1,2], L. Huan[1,2], A. Adebiyi[1,2], R. Aluko[1,2], [1]University of Manitoba, Canada, [2]Richardson Center for functional Foods and Nutraceuticals

The aim of this study was to determine the antihypertensive effects of hempseed protein hydrolysate (HPH) and its peptide fractions. Hempseed protein isolate (HPI) was sequentially digested with pepsin and pancreatin enzymes to mimic gastrointestinal digestion in human beings.The resultant HPH was separated via membrane ultrafiltration into peptides of different sizes (

Experimental Electron Density Distribution of 6,6′-dimethoxygossypol, a Gossypol Derivative Isolated from Cotton Plants.

C.A. Zelaya[1], E.D. Stevens[1], M.K. Dowd[2], [1]Dept. of Chemistry, University of New Orleans, New Orleans, LA, USA, [2]SRRC, ARS, USDA, New Orleans, LA, USA

Gossypol is a natural product of the cotton plant that is of interest because of its wide sphere of bioactivity. We have isolated and synthesized a number of gossypol derivatives to explore their anticancer and antifungal activity. Crystals of the 6,6′-dimethoxy derivative were found to be suitable for a high-resolution study of the structure's electron density distribution. A highly redundant set of X-ray diffraction intensity measurements was collected to $(\sin \theta/\lambda)_{max}$ of 1.18 $Å^{-1}$ at 120 K. The experimental electron density distribution was obtained by least-squares refinement of the scattered X-ray intensities by applying the Hansen-Coppens aspherical atom multipole model. In addition to maps of the molecular deformation density (i.e, the difference in density between the spherical and aspherical models), the topology of the electron density of dimethoxygossypol has been analyzed with the *Atoms in Molecules* approach. The location of the critical points in the density were found, and the electron density, the Laplacian of the density, and the bond ellipticity were determined at these points for the different types of bonds present in the structure.

Structural Changes of *Brassica napus* Storage Proteins during Commercial Oil Meal Processing.

W.G. Thushan Sanjeewa[1,2], Tara McIntosh[1], Janitha P.D. Wanasundara[1,2], [1]Agriculture and Agri-Food Canada, Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada

Pre-press solvent extraction (PSE) process dominates over any other oil recovery process used for canola. The key processing steps of PSE expose the disintegrated canola seeds to high temperature and pressure and non-polar solvents to harvest maximum amount of oil. During this process proteins and other chemical components may undergo several changes. This study investigated the structural changes occurred in canola seed storage proteins and how the key functional properties were changed during commercial PSE. Canola seed and meal samples obtained at the selected steps of commercial PSE processing line over a 3-month period were studied. Sampling points were where the drastic changes of temperature, pressure and solvent introduction occurred. Most of the changes to the proteins occurred in the processing step in which the material reached the highest temperature. The extractability of proteins even at strong

alkaline pHs reduced drastically as the processing progressed. Structure altered protein may be associated with other seed components that render them insoluble. Protein structure changes were evident as the 2° structure and peak thermal denaturation temperature changed along with processing. The need of alternative conditions for the solvent removal step seems necessary to make better use of the meal proteins.

Physicochemical Properties of Protein Isolates from Different Pea Cultivars.
A.K. Stone, N. Avramenko, T. Warkentin, M.T. Nickerson, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

The physicochemical properties of protein isolates from eight different pea varieties, cultivated on two different locations, were evaluated to better understand structure-function relationships. Specifically, the emulsifying properties, water hydration, lipid holding capacity, and solubility at pH 7.00 were examined and related to the protein′s surface characteristics. Isoelectric points for all protein isolates were found to be similar, ranging between pH 4.58 and 4.91 depending on the variety. Solubility differed significantly with variety, ranging from the highest solubility for CDC Dundurn (~75%) to the lowest for MFR042 (~54%). Varietal differences were also seen for water hydration and lipid holding capacities, which ranged between 1.72 to 2.7 g/g and 0.92 to 1.47 g/g, respectively; and for the emulsification activity and stability indices, which ranged between ~43.2 to 70.8 m^2/g and 10.2-11.7 (min), respectively.

Associative Phase Behaviour of Pea Protein Isolate and Alginate Mixtures.
K. Klemmer, L. Waldner, A.K. Stone, N.H. Low, M.T. Nickerson, University of Saskatchewan, Saskatoon, SK, Canada

Associative phase behaviour within admixtures of pea protein isolate (PPI) and alginate (AL) were investigated as a function of pH (1.50-7.00) and mixing ratio (1:1-20:1 w/w PPI:AL) by turbidimetric analysis and electrophoretic mobility during an acid titration. Critical structure forming events associated with the formation of soluble (pH_c) and insoluble ($pH_{\Phi 1}$) complexes in a 1:1 PPI-AL mixture were found to occur at pH 5.00 and 2.98, respectively, with optimal interactions occurring at pH 2.10 (pH_{opt}). As mixing ratios increased, critical values shifted towards higher pH until reaching ratios between 4:1 and 8:1, which were then constant. A similar trend was found for electrophoretic mobility, where there was a shift in net neutrality from pH 4.00 (homogenous PPI) to 1.55 for a 1:1 PPI-AL mixture. Solubility of the PPI-AL complexes at a 4:1 ratio showed: (1) improved solubility over PPI alone at pHs >4.00; (2) similar solubility at pH 4.00 (pI of PPI); and (3) reduced solubility at pHs