

2013 Annual Meeting Abstracts

Protein and Co-Products

MONDAY

AFTERNOON

PCP 1: Role of Protein and Co-Products on Food and Feed Supply and Security

Chair(s): J. Wanasundara, Agric & Agri-Food, Canada; N. Shah, Solae LLC., USA

Role of Protein and Co-Products in Food and Feed Supply and Security: An Overview

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Currently more than 850 million people globally are undernourished, and protein/energy malnutrition, the major concern, affects one quarter of children in the world. With the world population estimated to reach nine billion by 2050, 40 percent more food must be produced despite shrinking land and water resources. DuPont In collaboration with the Economist Intelligence Unit has developed a publicly available tool which defines and assesses food security in 105 countries (www.foodsecurityindex.eiu.com). This tool can be used to assess and to track improvements in food security in different regions over time. Science and technology have made large increases in food yields possible and this session highlights ongoing efforts to identify and leverage discoveries that can increase supplies of high quality protein. Identifying novel and/or lower cost animal feed and aquaculture ingredients and increasing plant-protein yield through novel processing technologies will help both human and animal feed supply constraints. The FAO will soon announce recommendations to change the method for determining the quality of dietary proteins to a measure called Digestible Indispensable Amino Acids Score (DIAAS). This talk will address how novel protein processing technologies and the FAO recommendations will impact research aimed to increasing high quality protein availability for human consumption.

Overview of pulse proteins on food and feed security

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Pulses are excellent source of energy, proteins, carbohydrates, dietary fiber, vitamins and minerals; and provide functional benefits for food and feed ingredients. Recent research indicated that pulses lower cholesterol, triglycerides and blood pressure; pulses lower blood glucose, fasting insulin levels and insulin resistance; and reduce hypertension and the likelihood of obesity. Pulses are processed with various processing technologies and pulse ingredients such as proteins, starches, fibers and flours are produced. Pulse proteins have variable protein levels (55-85%) and provide excellent nutritional, functional and cost reducing benefits for food & feed industry. In this presentation the impact of pulse proteins will be discussed in beverage, baked good, extruded snack, pasta, bread and feed systems; and global impact of pulse ingredients will be elaborated on food and feed security.

Feed Protein Ingredients - Conversion in to high quality safe food.

T. Scott⁽¹⁾

⁽¹⁾University of Saskatchewan, Canada

The feed industry is an important user of high protein ingredients, generally associated with legumes and co-products from the animal industry (e.g., meat and bone and blood meals), plant oil extraction (e.g., soybean and canola) and biofuel (distillers dried grains with solubles; DDGS) industries. A number of other sources of protein sources have potential, including new crops (e.g., camelina and carinata), algae, single cell protein and insects (e.g. maggots, locust, earthworms); most of these still require regulatory acceptance as safe and efficacious feed ingredients. Challenges that the feed industry faces are related to consistency of nutrient level and availability and the functional characteristics the protein source imparts to the finished feed. There are also factors that are considered, including level and type of anti-nutritional factors (e.g., enzyme inhibitors, allergens and associated chemicals such as tannins, phytate and toxins) or alternatively the presence of bioactives with nutraceutical value (e.g., bioactive peptides). With respect to nutrient level and availability variability, the industry is adapting rapid assessment technology to accurately predict and adapt rations using other ingredients and purified amino acids and/or altering process conditions or the addition of additives, such as enzymes (although proteases have not been utilized extensively to date). Due to increasing cereal costs (i.e., due to competition for direct use as food, biofuels and most recently wide spread drought) it is becoming more difficult to economically balance protein and energy in animal diets. As a consequence, modifications to protein sources to increase their energy value are being sought.

Processing Effects on the Detection and Antigenicity of Priority Food Allergens

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Proteins are important components of food, supplying nitrogen and amino acids essential for health and growth. For up to 6% of children and 4% of adults, consumption of certain proteins can result in serious allergic reactions. Major food allergens include egg, milk, peanut, tree nuts, soybean, seafood (fish, crustaceans and shellfish), gluten containing cereals, sesame and mustard. To date the only effective risk management tool for allergic consumers is avoidance of allergenic and contaminated foods. Control of food allergens along the food supply chain is thus necessary to minimise the risk of undeclared allergens in foods. Labelling regulations in various countries require that allergens in foods be clearly declared either in the ingredient list or in a precautionary statement (e.g., ?may contain?). A major challenge in identifying and quantifying allergens when present in foods is the effect of processing on allergen detection and recovery. In the present work the effect of various processing treatments including high pressure, extrusion, heating, baking and irradiation on the structure and recovery of allergenic proteins in several different food matrices were studied. Results to be presented clearly showed that processing markedly impacts allergen detection with recoveries as low as zero obtained under some processing conditions.

Concentration and Characterization of Soy Whey Proteins from Isolated Soy Protein Waste Streams

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Typical isolated soy protein (ISP) processes only convert 44% of the incoming feed material (defatted soy flakes) into

commercial products. A significant percentage of the process streams are disposed of via costly waste water treatment operations. A process was tested for the separation and concentration of ISP whey to create sustainable new product combinations of protein, soluble carbohydrates and minerals. ISP whey is defined as the final separation supernatant from the acid precipitated ISP process. ISP whey contains protein, mineral, and carbohydrates. Soy whey protein (SWP) is primarily comprised of those proteins purified and concentrated from the ISP whey. The current process consists of several concentration and separation steps, including membrane filtration and centrifugation. The material is then spray dried, screened, and optionally blended with soy oil or lecithin to reduce dusting. The unit operation tests generated samples with protein content exceeding 80% (dry basis) for the SWP and had a unique amino acid composition, which will be presented. These SWP samples showed unprecedented acid solubility (>90%) across a range of pH (3-6), low viscosity (with minimal shear impact), emulsion interfacial tension properties similar to sodium caseinate, and foaming capability unmatched by dairy proteins. The potential opportunities to create new healthy ingredients from SWP will be proposed.

The importance of structure-function relationships to a better understanding of food-related enzyme functionality: Aspartic proteases as a model system

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Enzymes play major roles in many food products including acting as processing aids (e.g., rennin), as antimicrobials (e.g., lysozyme) and in the quality of foods (e.g., polyphenoxidase). However, a better understanding of their structure-function relationships is critical to their efficient use and/or design for optimal activity under given conditions of usage. Aspartic proteases have traditionally been used in a number of food applications, and from a structure-function perspective, they constitute an ideal group of enzymes to study as they share similar conformations and active site configurations (i.e., aspartic acid residues), but also deviate in structure and ranges of functionalities. Using molecular biology approaches and advanced technologies (e.g., atomic force microscopy, solution NMR), our group has used aspartic proteases as a model system in order to introduce some novel functionalities (e.g., increased thermostability, antimicrobial activity) as well as to better elucidate other structure-function (e.g., antifungal) relationships. Some of our recent structure ?function work regarding various food-related aspartic proteases (e.g., pepsin, potato AP) will be discussed.

Manufacture of Low cost Soy Protein Concentrate for Aquaculture and other Feed Markets

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Soy Protein Concentrates have long been used in the Food Additive Marketplace as protein supplements and functional additives. In the past, these products have been too expensive for extensive use in the Aquaculture Marketplace. With the worldwide decline of the supply of fishmeal and the increase in demand for low cost protein for human consumption (i.e. farmed fish) the demand for vegetable based proteins has increased rapidly. Recently, processing methods have been developed that decrease the cost of soy proteins making it possible for use in the Aquaculture marketplace. This paper will discuss the benefits of Soy Protein Concentrate for the Aquaculture marketplace and processing alternatives for the manufacture of SPC. We will also talk about the possibility of using other oil seeds in the Feed Marketplace.

Enriching soy Proteins From Meal by an Enzymatic Approach

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Soybean meal, on dry basis, contains about 50% proteins and 40% carbohydrates, the latter include soluble carbohydrates and insoluble dietary fiber consisting of cellulose, hemicellulose and pectin. For many applications it is desirable to increase the protein contents in soy-based products. Currently, two levels of commercial products are available: soy protein concentrate with 65-70% proteins, and soy protein isolate with 90+% proteins. We have been developing an alternative enzyme-based approach for obtaining enriched soy proteins. The enzymes used were produced in our laboratory by fermentation with different species, inducers, and operation conditions. The enzymes produced were evaluated in the enrichment process and the results were used for optimizing the producing fermentation and the enzyme dosage and conditions for enrichment process. The developed enzyme-based process gave two groups of soy protein products with 90+% total protein recovery. One group had 70-80% proteins; the other had 91-96% proteins. Results of this work will be presented and discussed.

TUESDAY

MORNING

PCP 2: Protein and Co-Products of Algae

Chair(s): R. Green, POS Bio-Sciences, USA; S. Chen, Washington State University, USA

Polysaccharide as Co-product from Algal Biomass

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Excessive amount of energy required to dry the algal biomass is a major limitation for using traditional solvent extraction method to extract the lipid from algae cell. We have developed a sequential hydrothermal liquefaction technology to fractionate different components of algal biomass. At lower temperature, protein and polysaccharides were separated first followed by liquefaction of the remaining residue to bio-oil at higher temperatures. This presentation summarizes the results of physicochemical characterization of the extracted polysaccharide from *Chlorella sorokiniana*. Structural and chemical characterizations of crude polysaccharide were determined using different spectroscopic analyses. Monosaccharide composition and linkage analysis revealed that the >90 % of the polysaccharide is composed of 1?4 linked glucan. As quantified based on molecular weight cut off of the dialysis bag, 68-70% of the ethanol insoluble polysaccharide showed to have a molecular weight >10,000g/mol. The polysaccharide exhibits pseudoplastic behaviour at 0.05 g/ml and can maintained over a NaCl concentration of 0.1 to 3 M. Due to its simple chemical structure and ?-1-4 linkage the polysaccharide can easily be hydrolysed. The sugar produced can be recycled back for algae biomass production or for use for other purposes. Furthermore, pseudoplastic behaviour along with its low molecular weight provides a scope to explore the potential use of this polysaccharide in various industrial products e.g. in fire extinguisher as Aqueous Film-Forming Foam (AFFF) concentrates, in developing low molecular diester cross-linked hydrogel for multiple uses e.g. in cosmetic, and in surgery as bio-adhesive etc.

Potential co-products to the algae oil process

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In recent years there has been a strong increase in the utilization of microalgae as a lipid source for food and fuel. Although supported by significant research and development activity, the technology particularly when related to the production of biofuel and co-products is still in its infancy. Besides the desired lipid based end products, microalgae contain a range of potential valuable components such as; surfactants, proteins, carbohydrates and hydrocolloids, phytosterols, carotenoids and other secondary plant metabolites. The type of organism, lipid extraction and refining conditions, desired product specifications and processing design all affect the feasibility to commercialize these value added streams. In this presentation an overview of the limited research performed on these compounds sourced from algae will be provided, particularly related to the protein and carbohydrate fractions. Furthermore, the effect of upstream processing conditions on these compounds will be discussed.

A research summary: Post-extraction algal residue as a protein supplement to cattle consuming low-quality forage

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Sustainable algal biofuel production is partially dependent on placing coproducts in markets of optimal scale and value and effectively capturing those nutrients in a valuable form. Beef cattle are documented users of coproducts, produce a source of high-quality protein for human consumption, and often consume basal diets deficient in protein and not directly consumable by humans. Therefore, we conducted four studies to determine the potential for post-extraction algal residue (PEAR) to be incorporated into protein supplements for beef cattle. Our first three experiments were designed to evaluate PEAR's palatability, to its compare effects on nutrient utilization versus a conventional protein supplement, and its impact on nutrient utilization. Our final project quantified the effect of upstream processes on PEAR's nutritive value. Observations indicate PEAR may be blended with existing protein sources in the beef industry without negatively affecting palatability, but potential palatability concerns exist when PEAR is offered alone. Provision of PEAR or CSM at isonitrogenous levels stimulated forage intake ($P < 0.05$) and increased N retention ($P = 0.02$) relative to unsupplemented animals. Imbalances in mineral intakes (Ca:P ratio of 8:1) were observed when PEAR was supplemented, but not CSM. Forage utilization at isonitrogenous levels of PEAR and CSM was not different ($P > 0.13$). Excess mineral levels and imbalances in PEAR resulted from differences in cultivation, harvesting, and extraction procedures. Overall, our results suggest PEAR can be incorporated as a protein source in the beef cattle industry, likely increasing the economic and environmental sustainability of biofuel production from algal biomass.

Panel Discussion and Q&A

PRO 2/PCP 2.1: Hexane-Free Oil Extraction (Green Extraction)

Chair(s): F. Temelli, University of Alberta, Canada; D. Balke, BioExx Specialty Proteins Ltd., Canada

Recent Advances in Enzyme-assisted Aqueous Extraction of Soybeans

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Concerns over safety and pollution are driving alternative soybean processing technologies. One such alternative is aqueous extraction. When soybeans are ground in water, a small amount of free oil and a larger amount of oil-rich cream float on a protein- and sugar-rich solution (soy skim) retaining small amounts of emulsified oil. The fiber settles out. After centrifuging, 45% of the oil is recovered as a stable cream emulsion. The skim contains 19% of the oil, most of the sugars and 92% of the protein. Lower oil yields and dilute protein streams unsuitable for feed remain challenges. Many advances have been made including: cracking, dehulling, flaking and expanding to rupture cell walls and pseudomembranes around oil bodies (improves oil extraction to 71%); employing protease to enhance oil extraction (improves oil extraction to 96%); employing two countercurrent extraction stages (improves oil extraction to 99% and protein extraction to 96% while reducing water by 40%); using protease in the second or both stages to produce proteins with different functionalities; breaking the cream emulsion using the same protease as used for extraction; recycling enzyme from cream demulsification into extraction; recovering protein by isoelectric precipitation when using enzyme in the second stage only or by membrane filtration when using enzyme in both stages; and using soy skim to slurry corn for fermentation in a soybean-corn biorefinery. When integrating these advances the process is known as enzyme-assisted aqueous extraction processing and 98% oil extraction, 79% oil recovery and 97% protein recovery are possible.

The Development of a 'Green' Aqueous Enzymatic Process to Extract Corn Oil From Corn Germ

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We developed an aqueous enzymatic oil extraction process that uses cellulase and it results in oil yields of greater than 90% from wet milled corn germ and from E-germ. We also developed a second process that uses a combination of an acidic cellulase and an alkaline protease and it results in oil yields of 50-70% from dry milled or dry fractionate corn germ. Experimentation is underway to increase these oil yields. Both processes do not involve the use of hexane or any other organic solvents. Today, more than half of the ~200 dry grind ethanol plants in the US are removing oil (for use as a biofuels and/or animal feed ingredient) after fermentation and distillation at the 'back end' of the plant. Removal of corn germ at the 'front end' of dry grind ethanol plants and extraction of the oil using an aqueous enzymatic oil extraction process could potentially result in higher oil yields and higher value oil.

Review of the current status of enzymatic aqueous extraction processing in China

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Commercial oils today are mainly extracted using organic solvents, however, the organic solvents have environmental and safety problems, and, due to the increased awareness of safety and environmental issues, it is possible to develop alternative methods to organic solvent extraction. The enzymatic aqueous extraction processing (EAEP) method as an alternative approach to the conventional organic solvent extraction method has been widely applied to extract oil from several seeds. In China, numerous studies were carried out aimed to extract oil from several varieties of seeds using EAEP method, such as perilla seed, hemp seed, tea seed, hazelnut seed, etc.; and studies aimed to de-emulsify the cream layer from EAEP, thereby improving the oil extraction yield. So on and so forth, the EAEP method gets faster development in China. This review summarized the current status of EAEP method in China.

Recovery of oil and saponin from *Camellia oleifera* seeds by aqueous enzymatic extraction

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Camellia oleifera is native to China and its seeds have high contents of oil rich in oleic acid and many natural antioxidants with various biological activities and contain saponin. Aqueous enzymatic extraction (AEE) is a safe and efficient vegetable-oil extraction process that may also result in value-added by-product. In this research, the chemical characteristics of *Camellia oleifera* seed kernel were determined in order to design and evaluate studies on aqueous enzymatic extraction oil and sacchariterpenin from the kernels. The pretreatment of the materials and the effectiveness of a number of cellular degrading enzymes were tested by treating meals of the kernels with one or more of these enzymes and comparing the yields of free oil and sacchariterpenin. And the best enzyme was selected with higher oil yield and non emulsion layer in the processing chart. Through the single factor experiment and optimization experiment, the optimum technological conditions are determined and the free oil and thea saponin yields were 89.17% and 81.20%. The liquid phase containing saponin and carbohydrate were recycled by ceramic membrane and can act as feed additive sacchariterpenin.

Developing Sustainable Oilseed Extraction Using Extended-Surfactants

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The vegetable oil extraction industry is the primary contributor of volatile organic compound emissions in the food industry in the U.S. The annual hexane loss in the soybean oil extraction process could be as high as 210 ? 430 million liters. There are growing health concerns and increased environmental regulations regarding the use of hexane in vegetable oil extraction. The U.S. Environmental Protection Agency (EPA) established regulations on hexane emission due to growing environmental concerns. There is a pressing need for more sustainable extraction method as an alternative to hexane. This presentation will give an overview of existing alternative oilseed extraction technologies and discuss the advantages and disadvantages of these methods. We will next present our pioneering research work on aqueous-surfactant-based extraction method for oilseed extraction. With the advancement in the surfactant science, a novel class of surfactant, so-called extended-surfactant, which has an intermediate polarity groups inserted between the head and tail of a surfactant molecule, is able to produce ultralow interfacial tension with a wide range of vegetable oils at ambient temperature within a reasonable time frame. At optimum extraction conditions, we achieved more than 90% extraction efficiency for peanut and canola oils at 25oC in both batch and semi-pilot scale study using desirable operating conditions. The oil quality produced from the aqueous extended-surfactant based method was found to be comparable or even superior to that obtained from hexane-based extraction, further demonstrating the viability and sustainability of aqueous extended-surfactant based extraction.

Isopropyl Alcohol Extraction of De-hulled Yellow Mustard Flour

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Fossil fuels represent a significant source of worldwide energy consumption. The search for alternative sources of energy is a necessity. Vegetable oils which have an energetic content close to fossil fuels are an example of a renewable and potentially inexhaustible source of energy. An annual production of almost 160,000 tonnes of mustard (2008) makes Canada the world's largest exporter of mustard seed. Mustard seed oil contains erucic acid which is known to contribute to certain heart conditions such as myocardial lipidosis. As a result, in Europe and North America mustard oil is banned for human consumption. It is also known that the presence of erucic acid in mustard seed oil is responsible for its high lubricity, a positive property of bio-fuels. As a result mustard seed is a good candidate for production of biodiesel. Investigation of oil recovery from de-hulled yellow mustard flour using isopropyl alcohol (IPA), as the solvent, has been performed. This investigation helps current understanding of IPA extracted mustard oil composition which affects the transesterification process for biodiesel production. IPA is a polar solvent therefore some non-acylglycerol material may also be extracted along with the oil, such as phosphorus, water, carbohydrates and protein. The composition of the miscella (IPA+Oil) and meal residue resulted from extraction has been determined. In addition to oil, IPA was shown to have significant affinity towards water and some affinity to carbohydrates. The effect of water content of the IPA on the components dissolved in the solvent was further investigated.

Ultrahigh Pressure Supercritical Fluid Extraction ? Déjà Vu

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Recent research and development activity in supercritical fluid extraction (SFE) has seen the increase use of extraction pressures above 700 bar, thereby resulting in extracts that are more representative of the botanical source matrix and having critical constituents showing bioactive properties and health benefits. By conducting extractions up to 1000 bar in pressure with CO₂, the enhanced extraction of more difficult to extract components having higher cohesive energies, polarity and molecular weights is realized, resulting in a superior product containing ingredients up to twenty-fold higher in concentration relative to those obtained at much lower pressures. By optimizing the size of the extraction vessels in the pilot or production plant, costs for such plants become comparable to those operating at lower extraction pressures. These "new" results will be compared with unpublished studies of Friedrich and colleagues at the USDA involving SFE over the pressure range of 650-1025 bar in which vegetable oil and natural product extracts exhibiting different tinctorial properties were obtained. The physicochemical basis of the resultant extracts will be rationalized by comparing the reduced density and solubility parameters of SC-CO₂ with those solutes undergoing extraction enhancement. SFE results will be presented for commodity and specialty seed oils, natural pigments, and some spices. These results as well as more recent data for the enhanced extraction of fatty acids, sterols, vitamins, and pigments from seed oils and cakes, algae, as well as other botanicals will be used to illustrate the value of this approach.

Supercritical Carbon Dioxide Technology as Part of a Biorefinery: A case for the Processing of Distillers Grains

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Supercritical carbon dioxide (SC-CO₂) technology has been established as an efficient and "green" technology for the extraction of lipids from a variety of natural materials. To take full advantage of the benefits of SC-CO₂, incorporation of SC-CO₂-based operations into a biorefinery shows great potential to maximize utilization of biomass resources, targeting both food and industrial products. A case study will be presented to demonstrate that SC-CO₂ can be used not only for the extraction of lipids from dried distillers' grains with solubles (DDGS), but also for the continuous bioconversion of the extracted lipids to fatty acid methyl esters (FAME) to be used as biodiesel in two integrated operations. DDGS was a good inexpensive source of lipids and valuable minor lipid components such as carotenoids,

tocols and phytosterols. SC-CO₂-extracted DDGS lipids were converted into FAME in a continuous SC-CO₂ bioreactor using immobilized lipase. Supercritical process yielded FAME up to 95%, and it did not have any inhibitory effect on the immobilized enzyme. Continuous bioconversion of DDGS lipids to FAME in SC-CO₂ is a simple, efficient and "green" alternative to the conventional processes. Such an integrated supercritical approach to lipids processing, avoiding the use of organic solvents, fits well into larger biorefineries.

AFTERNOON

PCP 3: Protein and Co-Products of Hexane-Free Oil Extraction

Chair(s): S. Jung, Iowa State University, USA; N. Deak, Solae LLC., USA

Recovery and functionality of soy protein produced by countercurrent two-stage enzyme-assisted extraction

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Enzyme-assisted aqueous extraction processing (EAEP) is an environmentally friendly technology where oil and protein can be simultaneously extracted from soybeans by using water, enzyme, and mechanical treatments. The significant amount of protein-rich effluent (skim) constitutes a challenge to protein recovery. Protein recovery strategies using a two-stage cross-flow membrane filtration (RC 30 Kda and PES 10% NaCl rejection) and isoelectric precipitation followed by whey nanofiltration (PES 10% NaCl rejection) were evaluated in recovering protein from skim and whey produced by the countercurrent integrated two-stage EAEP of soybeans at pilot plant scale. Skim, curd, retentate and permeate proteins were analyzed regarding solubility, foaming, and emulsification properties.

Use of protein from enzyme assisted aqueous extraction of soybeans for use as fermentation aid

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Proteins obtained from an enzyme aqueous extraction of soybeans versus traditional hexane/pressure methods are theorized to have more functionality and enhanced uses over the material from conventional methods. Previous authors have reported on functional properties being improved. The purpose of this study was to evaluate the protein from this new aqueous method for feasibility as a protein source for industrial fermentations. Data obtained will show that this "new" protein is capable of being used in industrial fermentations and can be as effective at being a nitrogen source as traditional soybean protein. The benefits of this material are in providing an additional revenue stream and justification for scaling up this new technology along with improved economics for industrial products such as probiotics, enzymes and pharma products.

Increase feed quality through fermentation of corn and soy processing co-product

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The aim of the study was to use fungi fermentation to improve the composition of DDGS, soy fiber obtained from the optimized soybean aqueous processing, and the soy enhanced DDGS which is the residue after using soy skim as corn fermentation medium. The effect of three different fungi, *Aspergillus oryzae*, *Trichoderma reesei*, and *Phanerochaete chrysosporium* on enzyme production by solid-state fermentation (SSF) was studied. When soybean fiber was used as the substrate, maximum xylanase activity of 757.4 IU/ g and cellulase activity of 3.2 IU/ g were achieved with the inoculation and incubation of *T. reesei* and *P. chrysosporium* for 36 h, followed by *A. oryzae* for additional 108 h. This inoculation scheme also resulted in the highest xylanase activity of 399.2 IU/g compared to other fungi combinations in the SSF of DDGS. A large scale SSF by this fungi combination produced fermented products that had 3.5-15.1% lower fiber and 1.3-4.2% higher protein contents, suggesting a potential feed quality improvement. We have shown that arachidonic acid (ARA) and eicosapentaenoic acid (EPA) can be produced by *Pythium irregulare* fungus fermentation using soybean cotyledon fiber and soy skim as substrates. Parameters such as moisture content, substrate glucose addition, incubation time, and vegetable oil supplementation were found to be important in solid-state fermentation (SSF) of soybean fiber. Soybean fiber with 8% (dwb) glucose supplementation for a 7-day SSF produced 1.3 mg of ARA and 1.6 mg of EPA in one gram of dried substrate. Soy skim was shown to be a good medium too.

Characterization of the Functional Attributes of pea Protein Isolates Prepared Using Different Extraction Methods and Cultivars

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The functionality of pea protein isolates was investigated as a function of extraction method [salt extraction (SE), isoelectric (IEP) and micellar precipitation (MCP)] and cultivar [CDC Striker, Meadow and Dakota]. Specifically, water (WHC) and oil holding (OHC) capacities, solubility, foaming and emulsifying properties were assessed. SE and IEP gave higher extraction efficiencies (~63-77%) than MCP (~31%), independent of cultivar. Protein levels for isolates prepared by IEP, SE and MCP were ~83-87%, ~73-79% and ~82-88%, respectively regardless of the cultivar. Overall, WHC was greater for isolates prepared by MCP, followed by IEP and SE; whereas WHC for Dakota and Meadow were similar, and higher than for Striker. Overall, OHC was greater for isolates prepared by SE than MCP or IEP, regardless of the cultivar used. Protein solubility was greatest for isolates prepared by SE, followed by IEP and then MCP, where no effect of cultivar selection was observed. Foaming capacity was found to be greater for isolates prepared by SE, followed by IEP or MCP. However, overall Dakota showed greater foam forming properties, followed by Striker and then Meadow. Foam stability was found to decrease in isolates prepared by IEP, then MCP followed by SE. Also, foam stability was overall lower for Dakota, relative to Meadow or Striker. Overall, emulsion capacity was greater for isolates prepared by SE than IEP; and for Dakota followed by Meadow and Striker. Isolates prepared by MCP did not form oil-in-water emulsions. Emulsion stability was similar among methods of production and cultivar.

Phenolic Compounds and Antioxidant Activity of the Solid Residue of Cold-pressed Brazil nut (*Bertholletia Excelsa*) Oil

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The solid residue (cake) from cold-pressed Brazil nut possibly retains bioactives originally present in this oilseed, such as phenolics. The aim of the present study was to evaluate the contents of phenolic compounds and antioxidant capacity of the Brazil nut cake. Brazil nuts were cold-pressed in a continuous screw-type laboratory-scale press (OEKOTEC, Germany), and the cake was used for all analyzes. Antioxidant compounds were extracted from the

Brazil nut cake with ethanol:water (40:60, v/v), and the content of total phenolic compounds was determined by the Folin-Ciocalteu assay, and expressed in gallic acid equivalents (GAE)/100 g. The antioxidant capacity was determined by FRAP, ORAC and TEAC assays. The Brazil nut cake presented (mean \pm SD) 21.14 \pm 0.23 moisture, 22.29 \pm 2.34 protein, 31.72 \pm 1.11 lipids, 5.33 \pm 2.60 ash and 182.4 \pm 0.10 mg GAE/100 g of total phenolic compounds. The antioxidant activity of the Brazil nut cake was 653.1 \pm 0.08 μ mol Fe²⁺/100 g, 151.5 \pm 0.4 μ mol trolox equivalents (TE)/100 g and 0.407 \pm 0.03 μ mol TE/100 g, for the assays of FRAP, ORAC, and TEAC, respectively. Our results show that Brazil nut cake presents extractable contents of phenolic compounds with antioxidant activity, and therefore might subsidize future developments of food products from the Brazil nut cake.

Protein in Wet-milled Corn Germ Recovered by Ultrafiltration-Diafiltration

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⁽¹⁾USDA ARS NCAUR, United States of America

This study was conducted to evaluate ultrafiltration-diafiltration (UF-DF) as a means to improve the extractability of wet-milled corn germ protein and determine its effects on the functional properties of the recovered protein product. Wet germ and finished (dried) germ proteins were extracted by using 0.1M NaCl at 50°C. Major steps in the method were homogenization, stirring, centrifugation, UF-DF and freeze-drying. UF-DF increased protein extraction efficiency of wet germ while that of dried germ remained similar to what we observed for the baseline method (saline extraction without UF-DF). The recovered freeze-dried UF-DF protein from both germ samples showed the same unusual solubility profile; i.e., the amount of soluble protein was essentially unchanged under acidic, neutral, and alkaline conditions. However, UF-DF finished germ protein was markedly more soluble (80% soluble protein vs. 50% soluble protein in wet germ at pH 2-10). UF-DF protein extracts from both germ samples also had emulsifying capacities and water-holding capacities that were notably greater than those of germ protein recovered by the baseline method, but this favorable effect by UF-DF was not observed for emulsion stability and foaming properties.

WEDNESDAY

MORNING

PCP 4: Nutritional and Safety Aspects of Plant and Animal Proteins and Co-Products

Chair(s): H. Ibrahim, Kagoshima University, Japan; H. Kumagai, Nihon University, Japan; Y. Mine, University of Guelph, Canada

Effects of Glycoconjugates of Ovalbumin on Alleviation of Orally Induced egg Allergy in Mice

Y. Mine⁽¹⁾

⁽¹⁾University of Guelph, Canada

Glycation of allergens via Maillard reaction or chemical conjugation have been shown to influence susceptibility to food-induced allergies. We have investigated the efficacy of various glycated forms of Ovalbumin (OVA) as mucosal immune response polarizing candidates for the treatment of allergy in a Balb/c mouse model of egg allergy. Balb/c mice (n=10) were orally sensitized to OVA and subsequently administered various forms of glycated-OVA (glucose, mannose, glucomannan, galactomannan and a mixture containing OVA and glucomannan) followed by oral challenge with OVA. Clinical signs were less frequent in the OVA-glycated mannose and glucomannan treated groups (p \leq 0.05)

and significant decrease in specific IgE and increase in percentage of T-reg cells were observed with both groups. The OVA-glycated mannose treated group also had less histamine, MMCP-1, specific IgG, IL-4 and IL-17 ($p < 0.05$) and more IL-12p70 ($p < 0.001$). Other immunoglobulin isotype-associated antibody activity and cytokines measured did not differ significantly among groups. Also, OVA-glycated mannose reduced maturation and uptake by dendritic cells (DC). Activation of T-cells as measured by IL-2 concentration and type-2 cytokine response in treated DC-T-cell co-cultures was less with the OVA-glycated mannose stimulation. In conclusion, OVA-glycated mannose and glucomannan could reduce susceptibility to OVA-induced allergy.

Blood Pressure Lowering Effect of Gaba Enriched Salt-free Soybean Paste in Human Volunteer Test.

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Gamma-aminobutylic acid (GABA) is generated from glutamic acid by glutamic acid decarboxylase (GAD). GABA has several biological functions such as blood pressure (BP) lowering effect, brain metabolic activation, relieving anxiety, et cetera. Among these functionalities, BP lowering effect is the most attractive these days. According to the latest report, about 40 million people are hypertensive including borderline hypertensive in Japan. Therefore, controlling BP is very important by reducing salt intake as well as taking GABA enriched diet. We found and isolated a lactic acid bacterium (*L. hilgardii* K-3) from Korean pickles which has surprisingly strong activity of GAD, and by applying this bacterium in fermentation we succeeded in producing GABA enriched salt-free soybean paste which contains about 0.2% GABA. In this report, we prepared GABA fortified cookie by use of the GABA enriched salt-free soybean paste, and examined its BP lowering effect in single blind volunteer test. Ten adult volunteers in borderline hypertension (aged 22-56) were divided into control and test groups, and took either a control (GABA 2.1mg) or test (GABA39.4mg) cookie, respectively, per day for one month. BP of all volunteers was measured by themselves before breakfast of three consecutive days in every weekend during taking a cookie period and each 2 weeks pre- and post-the period. BP of the test group were significantly reduced by 5-20mmHg individually after taking GABA cookie for 2-4 weeks, however BP of the control group were not significantly changed.

Simultaneous Enzymatic Hydrolysis and Fractionation of Bioactive Peptides From Beta -lactoglobulin by Electro dialysis With Filtration Membrane

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⁽¹⁾Université Laval, Canada

The hydrolysis of β -lactoglobulin protein and the fractionation of generated peptides were performed in one step in an electro dialysis with filtration membrane (EDFM) cell. After 240 min of treatment, amongst the 30 peptides peaks detected in the β -lactoglobulin hydrolysate which corresponded to 30 peptides, respectively, 15 and 4 peptides were detected in the anionic and cationic peptide recovery compartment. Amongst these 15 peptides, 2 hypocholesterolemic peptides, 3 antihypertensive peptides and 1 antibacterial peptide were recovered and concentrated with migration rates ranging between 5.5 and 88.1%. Amongst the 4 cationic peptides, the peptide sequence ALPMHIR, identified as lactokinin and known to exert an important antihypertensive effect, was recovered and concentrated. At our knowledge, it was the first time that hydrolysis was conducted under an electric field to simultaneously separate anionic and cationic peptides produced.

Production of hypoallergenic rice by enzymatic treatment and analysis of rice-allergen epitopes

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Cereal allergy afflicts those who consume the cereal as a staple food. In Japan, several types of hypoallergenic rice have been developed for patients suffering from rice allergy. However, the manufacturing process required for their production is complicated. Therefore, this study aimed to develop a simple technique to reduce the allergenicity of rice by using enzymes. In addition, the epitope structure of rice allergens was analyzed. Rice grains were soaked in an enzyme solution and cooked. Proteins were extracted from the rice, and the allergenicity was evaluated using enzyme-linked immunosorbent assay and Western blotting. The synthetic peptides that have a high affinity for the 16-kDa rice-globulin specific antibody were reacted with the sera of 12 patients with rice allergy to determine the sequential epitope. Rice allergens with conformational epitopes were identified by using 2D-PAGE in which isoelectric focusing in the first dimension and blue-native PAGE in the second dimension were performed. Among the enzymes used, papain was found to be effective in reducing the allergenicity of rice without deteriorating its sensory properties. The peptide, GWCR, reacted the sera of all the patients. Several proteins, such as triosephosphate isomerase, were estimated to have conformational epitopes in their structures.

Brewers? Spent Grain (bsg) Protein Hydrolysates and Phenolic Co-products: Potential use as Ingredients in Functional Foods

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The present study investigated the antioxidant and anti-inflammatory potential of eight phenolic extracts from BSG; four pale (P1-P4) and four black (B1-B4). For assessment of antioxidant potential, U937 cells were pre-incubated with BSG extracts and exposed to hydrogen peroxide (H₂O₂) and antioxidant activity was measured by the superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) assays. An enzyme-linked immunosorbent assay (ELISA) was used to measure the immunomodulatory effects of the BSG extracts in concanavalin-A (conA) stimulated Jurkat T cells. The effect of the extracts on interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon- γ (IFN- γ) production was measured. Extracts P1-P3 and B2-B4 showed significant ($P < 0.05$) antioxidant effects by at least two antioxidant activity assays. Pale BSG extracts P2 and P3 showed greatest anti-inflammatory potential; P2 significantly ($P < 0.05$) reduced IL-2, IL-4, IL-10 and IFN- γ production and P3 significantly ($P < 0.05$) reduced IL-2, IL-10 and IFN- γ production. Phenolic extracts from BSG, particularly the pale BSG extracts, have the ability to protect against oxidative stress and reduce stimulated cytokine production. The antioxidant and anti-inflammatory potential of protein and peptide-rich fractions of BSG are also currently being evaluated

Novel Therapeutic Potential of egg Ovotransferrin

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In recent years it has been recognized that dietary proteins provide a rich source of biologically active proteins. Egg albumen is a valuable source of bioactive proteins with diverse structural entities and many of them offer tremendous opportunities for drug development and hope for the treatment of emerging human diseases. This work explores, for the first time, an approach to treat intracellular bacterial infections and overcome antibiotic resistance by using

ovotransferrin from egg albumin. Specifically, exploring novel antibiotic drug-targeting strategy, which heralding a fascinating opportunity for its potential candidacy as anti-infection therapy.

Eggshell Bioactive Molecules

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The avian egg is a packaging system which has been exquisitely designed by evolution to protect and nourish normal embryonic development. The egg consists of key components organized in specific compartments, which, once understood, can serve as the basis for development of novel nutraceuticals for human health or enhanced eggs for human consumption. The egg has two types of defenses, physical and chemical, which resist bacterial invasion and maintain the sterile, unfertilized table egg as a highly nutritious foodstuff. The eggshell, together with its cuticle and shell membranes, contains a proteinaceous matrix with potential pharmaceutical and therapeutic value. Recent proteomic and transcriptomic studies of the eggshell protein constituents and their genes have identified candidate bioactive molecules, including the eggshell-specific ovocalyxins and ovocleidins, which regulate mineralization or are involved in the innate immunity of the egg due to their antimicrobial activity against invading pathogens.

Therapeutic Function of Deamidated Gliadin in Wheat Allergy

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Gliadin is mainly responsible for wheat-dependent exercise-induced anaphylaxis. Deamidation could be used to reduce wheat allergenicity because some tandem sequence repeats having glutamine residues constitute the primary structure of IgE-binding epitopes in wheat gliadin. Our previous findings showed that deamidation of wheat gliadin increased its solubility in water, and reduced its allergenicity during digestion both *in vitro* and *in vivo*. However, the allergic reaction occurs after the allergens are absorbed from the small intestine into the blood. Therefore, in the present study, the *in vivo* allergenicity of deamidated gliadin was evaluated by using a mouse model of wheat allergy. After sensitization of mice by intraperitoneal injection of wheat gliadin, untreated or deamidated wheat gliadin was intragastrically administered. Then, the intestinal permeability, the expression of Fc γ RI on mast cells, and the histamine and gliadin-specific IgE levels in plasma were measured. The administration of untreated wheat gliadin to the sensitized mice enhanced the intestinal permeability, while that of deamidated wheat gliadin did not change it. In addition, the level of epitope peptides was much higher for mice administered untreated wheat gliadin than for those administered deamidated one. Oral challenge of deamidated wheat gliadin down-regulated the surface expression of Fc γ RI on peritoneal mast cells in mice of wheat gliadin allergy. Moreover, the levels of histamine and gliadin-specific IgE in plasma were lower for mice administered deamidated gliadin than for those administered untreated one. These results indicate that oral challenge of deamidated wheat gliadin decreased allergenicity in mice of wheat-gliadin allergy.

Production and Nutritional Properties of a Branched-chain Amino Acid-enriched Flaxseed Protein Hydrolysate

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The aim of this work was to convert flaxseed proteins into a protein hydrolysate that is enriched with branched-chain amino acids (BCAA). Flaxseed proteins were hydrolyzed using thermolysin and pronase followed by mixing with activated carbon, centrifugation and filtration. Amino acid analysis showed that the filtrate (a mixture of peptides) had 29% (w/w) content of BCAA and very low (1.11%) levels of aromatic amino acids (phenylalanine + tyrosine). A Fischer ratio (BCAA/aromatic amino acids) of 23.65 was obtained for the peptide mixture. Thus the peptide mixture may be used to treat patients with hepatic encephalopathy since protein hydrolysates with a Fischer ratio of 20 is the minimum required value. The protein hydrolysate contained mainly low molecular weight peptides (<4 kDa) as shown by gel permeation chromatography, suggesting high potential for absorption from the gastrointestinal tract. This high Fischer ratio peptide mixture exhibited antioxidant property by scavenging 2,2-diphenyl-1-picrylhydrazyl radical, superoxide radical, hydroxyl radical, and also by protecting linoleic acid from oxidation. In addition, the peptide mixture showed potential modulation of the renin-angiotensin system because of its ability to inhibit angiotensin I-converting enzyme, a principal causative agent of hypertension. Therefore, this high Fischer ratio peptide mixture could be used to formulate food products with multiple human health benefits during liver diseases, oxidative stress and hypertension.

Effects of Dietary egg White Peptide on Body fat Mass and Lipid Metabolism in Rats

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Egg white protein (EW) was previously suggested to decrease serum and liver triglyceride concentration in rats. In the present study, we examined how peptides derived from EW can affect body fat mass, and serum and liver lipid concentrations in rats. As experiment materials, we hydrolyzed EW and prepared egg white peptides (EWP-1) and its water-soluble fraction (EWP-2). Then we fed male SD rats with AIN-93G diets containing 0 (control) or 10% of either EW, EWP-1 or EWP-2 substituted for casein. After 4 weeks, the weight of mesenteric adipose tissue tended to be lowered by feeding of the EW, EWP-1 and EWP-2 diets, and the weight of the tissue in rats fed the EWP-2 diet was the lowest. Serum triglyceride concentration tended to be lower in rats fed the EW diet than in those fed the control diet. The protein-dependent effect was also observed in rats fed the EWP-1 and EWP-2 diets, especially in the latter. Liver triglyceride concentration was not affected by feeding of EW, EWP-1 or EWP-2. Since serum leptin concentration was decreased especially by feeding of EWP-2, the adipose tissue weight could be associated with the decrease of serum leptin concentration. The results suggested that egg white peptide, especially EWP-2, could be a factor for modulating body fat mass and serum triglyceride concentration in rats, although further experiments are required.

New Advances on egg Proteins and Their Potential for Food and non Food Uses

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The avian egg is a basic food of high nutritive value for humans all around the world. It's also a closed chamber for the extra-uterine development of the embryo and possesses a wide range of biologically active compounds to protect the embryo for its development (physical defense ensured by the calcified eggshell and molecules involved in the chemical defense). The recent development of functional genomics tools in association with the chicken genome sequence were major scientific advances leading to the identification and characterization of hundreds of new egg

components that were not previously identified. We will describe here, the most recent developments in egg biochemistry (proteomics), and molecular biology (transcriptomics), and the use of these data to perform integrated analysis of biological activities of egg components. We will describe how we examined protein sequences for specific domains to predict their potential function as biologically active candidates. These hundreds of newly identified proteins have been classified in different categories according to their role in the antimicrobial protection and in the fabric of the eggshell (natural resistant bioceramic). The newly identified proteins potentially present beneficial properties for industrial uses as food and non food applications.

Absorption of Glutathione and its Derivatives Into Human Blood After Oral Intake

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γ -L-Glutamyl-L-cysteinylglycine (Glutathione: GSH) has long history as supplement to improve liver function and skin whitening. However, previous studies have not detected increase of GSH in blood after intake of GSH. Therefore doubt has been cast on efficacy of GSH supplementation. The objective of the present study was to detect increase of GSH and its metabolites in human blood after intake of GSH supplement. Healthy human volunteers were ingested GSH prepared from torula yeast (KOHJIN, 5 g/60 kg B.W). Blood was collected from median vein of forearm into heparinized tube. Plasma was mixed with 3 vol. of ethanol. The supernatant and precipitate were extracted with 5% TCA-2% 2-mercaptoethanol. The extracts were derivatized with AccQ to improve resolution by RP-HPLC. GSH yielded AQC-GSH-2-mercaptoethanol⁺ ions by ESI-MS analysis. Contents of GSH, γ EC, and CG were determined by the precolumn derivatization with AccQ followed by LC-MS/MS analysis. In the deprotonized fraction of blood, no significant increase of GSH was observed, while CG increased significantly. On the other hand, GSH, γ EC, and CG in the protein fraction (ethanol precipitate) significantly increased after the ingestion. These facts indicate that GSH and its derivatives are absorbed into blood system and transported as conjugate of protein.

AFTERNOON

PCP 5: General Protein and Co-Products

Chair(s): R. Aluko, University of Manitoba, Canada; M. Dowd, USA

Novel approaches to improve the dough structure by changing wheat flour proteins complex

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High quality of bread strongly depends on the variety of technological factors, in particular, flour properties. The main objective of this research was to develop a new approach to enhance gluten quality and improving rheological properties of the dough. The selected additives increased bread nutritional value (higher protein content) yet did not influence its safe consumption. The choice of additives was based on sensitiveness of protein structure forming bonds to pH value and hydrogen bonds number. Therefore, organic acidity regulators and polyatomic alcohols as substances forming hydrogen bonds (e.g., glycerol) were chosen for investigation. The scientific hypothesis was built by means of system analysis methodology. In case of low pH values (pH<7) particular NH₂⁻, NH⁻ and imidazole groups of proteins are involved into H⁺ ions binding which leads to electric balance shift and enhances proteins aggregation ability. Under relatively low pH condition protein amino groups interact with OH⁻ functional groups of glycerol

resulting in in gluten secondary structure which is involved in β -layer formation and stabilized by intermolecular hydrogen bonds. However, protein amino groups at $\text{pH} > 7$ interact with acid anions yielding the reduction of proteins macromolecules packing density and aggregation ability, but proteins solubility increased. Herewith, gluten extensibility increased and elasticity decreased. Consumer studies of modified wheat flour breads with tomato paste, juice (apple, grape) with 0.2% v/w glycerol showed high organoleptic properties proving glycerol positive impact on the dough structure.

Canola Protein-based Thermoplastic Polymers

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Diversified use of meal co-products enhances economic value of Brassica oilseeds. Bio-fuel oilseed crucifers such as Brassica carinata and Camelina sativa need uses for meal proteins beyond animal feed. Whole seed protein isolates of Brassica napus (canola) containing both 11S and 2S proteins had produced thermoplastic polymers with poor mechanical and high water permeation properties. In order to understand the contribution of each Brassica seed protein type in such material generation, cruciferin (11S) and napin (2S) of canola were studied separately for thermoplastic polymer development. Thermo-compression processing parameters; temperature (110-160C), pressure (8280-21720 kg) and plasticizer (43-77% glycerol) for the production of bio-plastics were studied. Influence of process variables on thermal properties (glass transition temperature, melting temperature by DSC analysis), mechanical properties (tensile stress, young's modulus, elongation by a texture analyzer), water uptake, and microstructure of the thermo-compression moulded plastics were tested according to a Central Composite Rotatable Design (CCRD). The optimum process conditions obtained from Response Surface Analysis were 120C, 15000 Kg and 60% glycerol for the lowest water uptake and maximum tensile strength. All input variables showed a significant effect on the quality of final product. Glass transition temperature of plastics was 15-20% low compared to original proteins. Microscopic examination revealed that high processing temperatures enhanced the dispersion and integration of protein into the polymer. Napin based bio-plastics possessed higher tensile strength and better stability than cruciferin based bio-plastics. Therefore, in a whole seed protein isolate, the positive properties of 2S protein for thermoplastic generation are not highlighted.

White Flake Desolventization of Soybeans & Other Oilseeds

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This paper will discuss current methods of manufacturing De Oiled Soy White Flakes when starting with a hexane wet flake or collet the objective being to preserve the protein value and color of the end product. The paper will make a side by side analysis of the major methods of White Flake Desolventization (both direct & indirect) available today discussing energy costs, product quality, and other issues. The paper will also discuss common applications for the white flakes such as Soy Protein Isolate & Concentrate and bakery applications and how to adjust operation conditions to tailor products to these areas. Past experience with soy other oilseeds will also be discussed.

Antihypertensive activity of laying hen eggs

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There are concerns over egg consumption due to the presence of cholesterol in egg yolk. As a protein rich food commodity, our previous work showed that simulated egg digestion could yield protein fragments (i.e. peptides) with inhibitory activity against Angiotensin-Converting Enzyme (ACE), one of the key enzymes responsible for regulating blood pressure. The aim of the study was to determine its *in vivo* antihypertensive activity in spontaneously hypertensive rats. Fried whole egg hydrolysate at a dose of 1000 mg/kg BW could significantly ($p < 0.01$) lower the blood pressure in SHR. Wire myograph study reveals that the fried whole egg hydrolysate treatment improved vascular relaxation, through nitric oxide production within the vessel endothelial cells. Our TBARs assay using various tissues reveals that the fried whole egg hydrolysate could also significantly reduce the tissue level oxidation. This result indicates that the fried whole egg hydrolysate also exhibited antioxidant effect, which might contribute to the relaxation mechanism through nitric oxide synthase. Our study showed that consumption of cooked whole eggs may have benefits for the prevention and treatment of hypertension, a risky factor for cardiovascular diseases.

Optimization of Preparation of soy Protein Hydrolysates With Anti- β -amyloid ($a\beta$ 1-42) Peptide Aggregation Activity Using Response Surface Methodology

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Soy bean is the richest source of protein (~ 40%) among legumes and is grown as a pulse crop around the world. Soy protein isolate (SPI), has the highest protein content and wide range of health benefits. In this study we aim to evaluate the anti-aggregative property of alcalase hydrolysates of SPI. SPI was prepared from defatted soybean meal, with protein and lipid content of 48.2% and 0.8% respectively by alkaline solubilization (pH 9.5). Since the anti-aggregative properties against Alzheimer's β -amyloid peptide ($A\beta$ 1-42) depends on the peptide composition of the hydrolysates and the enzyme used, the effect of process conditions on SPI hydrolyzed with alcalase was investigated systematically using response surface methodology. Hydrolysis conditions for optimal β -amyloid aggregation inhibition were defined using the response surface model of fractional factorial design (FFD), steepest ascent design, and central composite design (CCD). It was shown that the anti-amyloid aggregative activity of soy protein hydrolysates could be controlled by regulation of five process conditions pH (5.5-8.5), temperature (30-50C), enzyme/substrate ratio (.1-1%), substrate/water ratio (15-25%) and time of hydrolysis (2-8hr) by FFD. The optimum conditions for hydrolysate (with anti beta amyloid aggregation activity) preparation was found to be pH=8, Time=1 hr, E/S=0.5%, Temperature= 50 C. This is by far one of the first studies to show that soy protein hydrolysates possess bio-activity against beta amyloid peptide found in patients with Alzheimer's disease.

Molecular Structure, Physicochemical Characterization and *in vitro* Degradation of Barley Protein Films

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Barley protein films were prepared by thermo-pressing using glycerol as a plasticizer. The combined effects of heating temperature and the amount of plasticizer interacted to determine protein conformation subsequently the properties of the film matrix. The film barrier and mechanical properties were systematically investigated using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), SDS-PAGE and protein solubility tests. These experiments demonstrated that heating treatment induced barley protein unfolding and then protein aggregation and the formation of covalent disulfide bonds to enhance film strength. Increasing the amount of plasticizer reduced protein denaturation and limited protein interactions, resulting in significantly improved film flexibility at the cost of reduced film moisture barrier property and tensile strength. *In vitro* degradation experiments demonstrated that barley films

were resistant in gastric conditions, yet can still be completely degraded by intestinal enzymes, and they possess low cytotoxicity to Caco-2 cells. The prepared barley films have potential for the development as delivery systems for gastric-sensitive bioactive compounds to the intestine for release.

Comparison Between Interfacial Proteins Coming From Native or Transformed Flaxseed Lipids Emulsions

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When oleoproteaginous seeds are crushed in water, oil-bodies are released. Their stability is mainly achieved by their membrane rich in phospholipids and amphiphile proteins, named oleosins. Interactions with other hydrosoluble proteins from the seed has also an important role. Flaxseed proteins with their high content in arginin can have a wide range of applications in nutraceutics and pharmaceutics. Interfacial proteins can also be used in formulation for cosmetics and detergents. Their extractability depends on the strengths of lipids-proteins and proteins-water interactions. If triglycerides are hydrolysed, the structure and stability of oleosomes is changed as the nature of surrounding tensioactive proteins. The extraction of interfacial proteins is realized with different protocols with soft (pH based) to tough (solvent and salt based) conditions. Differences in amino-acid content, zeta potential, surface properties and concentration can explain differences in purity and extraction yields between proteins from triglycerides and fatty acids based emulsions.

Enzymatic hydrolysis of spent hen proteins with gastrointestinal proteases released peptides with blood pressure-lowering activity

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The objective of this work was to evaluate the health-promoting potential of spent hen (SH), an underutilized protein-rich byproduct of poultry egg production. SH breast meat proteins (SHP) were isolated by alkaline solubilization and acid-induced precipitation. SHP was then hydrolyzed with pepsin, pancreatin, and a combination of the proteases to simulate gastrointestinal hydrolysis. The resulting hydrolysates inhibited the activities of the enzymes of the renin-angiotensin system (RAS), the primary pathway that regulates physiological blood pressure and vascular tone. Specifically, the SHP hydrolysates inhibited angiotensin converting enzyme and renin by 81-84% and 23-38%, respectively, compared to the unhydrolyzed proteins, which displayed weak inhibitory activities of 31% and 16% against ACE and renin, respectively. The SHP hydrolysates generated with pancreatin generally displayed the best *in vitro* activity in inhibiting both RAS enzymes. When administered by oral gavage to spontaneously hypertensive rats (SHR) at a dose of 200 mg/kg body weight, the pepsin and simulated gastrointestinal hydrolysates induced decreases in elevated systolic blood pressure (SBP) by -27 and -36 mmHg, respectively after 2 h compared to the activity of captopril (10 mg/kg body weight), which had a Δ SBP of -11 mmHg. The antihypertensive effects of the hydrolysate samples were reduced by >75% over the next 6 h as opposed to the effect of captopril which increased by 100% over the same duration. All hydrolysate samples and captopril lost their activities after 24 h. These results indicate that spent hen proteins can be explored as starting materials for the production of antihypertensive peptides.

Effects of Fermented Rapeseed Meal on Antioxidant Functions, Serum Biochemical Parameters and Intestinal Morphology in Broilers

A. Li⁽¹⁾

With higher content of protein and sulph-containing amino acids, the utilization of rapeseed meal (RSM) is still limited. Solid-state fermentation (SSF) has been considered to be one of the best methods that can improve the qualities of RSM. To determine the effects of SSF on antioxidant functions, serum biochemical parameter and intestinal morphology of rapeseed meal in broilers. *Bacillus subtilis*, *Candida utilis* and *Enterococcus faecalis* were used as the microbe strain. A total of 180, male Arbor Acres broiler chicks were divided into three groups: Control, RSM and FRSM. The blood and intestinal tissue sections were collected and analyzed. With a higher level of total serum proteins on day 21, the serum T-AOC and T-SOD levels of birds fed by FRSM were superior to those fed by RSM. And the levels of serum albumin, uric acid, triglyceride and glucose of broilers by FRSM were significantly higher than those by RSM or the control. On day 42, a marked improvement in serum albumin was observed in FRSM group. With had significantly increased villus height. FRSM group had lower levels of serum total cholesterol than the RSM and control groups. In FRSM group, the levels of serum urea nitrogen were evidently poorer than RSM group and serum triglyceride level were lower than the control. Besides, the jejunum and cecum crypt depth were markedly lower than RSM group. Solid-state fermentation novelly designed in this study significantly reduced the anti-nutritional factors content and improved the nutritional quality of the rapeseed meal.

Protein and Co-Products Poster Session

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

CHANGES IN PROTEIN-RELATED FUNCTIONAL PROPERTIES OF LENTIL DUE TO MICRONIZATION

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Lentil is a high quality source of protein and a widely consumed legume around the world. Besides consuming whole lentil, developing lentil-based ingredients suitable for various food processing streams widens the use of lentil. Micronization is an infrared heat treatment that is used in pulse processing to reduce cooking time and enhance storability of whole pulses. Effect of lentil seed moisture levels due to tempering (three levels; natural 8%, or adjusted to 16 and 23% moisture content) and final temperature upon micronization (115, 130, 150 and 165° C) on the functional properties of resulting flour was investigated in this study. These moisture heat treatments modified functional properties of the resulting flour because of changes that occurred in proteins and carbohydrates. Protein dispersibility index of lentil flours decreased as the micronizing temperature increased for all tempering levels. Decrease in lipoxigenase, and peroxidase activities, trypsin inhibitory levels, protein enthalpy values of micronized treatments indicated substantial modification of the protein fraction. Liquid (water and oil) holding ability of the flours also increased due to micronization and these were sensitive to starting moisture level of the seed. Micronization brings physical modification of the lentil protein fraction that can enhance functionalities of the flour required in food product applications.

Effect of germination and fermentation on physico-chemical and nutritional properties of yellow pea (*Pisum sativum* L.)

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Pulses are emerging ingredients of interest due to their health benefits. Pre-treatments are necessary prior to consumption of pulses to remove anti-nutritional factors (e.g., trypsin inhibitors). This study explores the impact of fermentation and germination on the physico-chemical and nutritional properties of processed yellow pea seeds. The samples studied included raw yellow pea flour (RYPF), soaked yellow pea (SYP), soaked germinated yellow pea

(SGYP), soaked fermented yellow pea (SFYP), soaked fermented pasteurized yellow pea (SFPYP), soaked germinated fermented yellow pea (SGFYP), and soaked germinated fermented pasteurized yellow pea (SGFPYP). All treatments resulted in significant reduction ($p < 0.05$) in trypsin inhibitor activity ranging from -35% to -80% compared with the raw flours. The SFYP, SFPYP, SGFYP, and SGFPYP exhibited significantly increased ($p < 0.05$) water holding capacity and decreased endothermic parameters as measured by differential scanning calorimetry. In vitro protein digestibility ranged from 83.40 to 87.92%, with samples such as SYP, SFPYP and SGFYP showing significantly enhanced values in comparison with RYPF. The protein profile of the raw and processed pea flours as studied by SDS-PAGE and SE-HPLC, showed a rich composition of proteins having MWs of ~50 and ~65 kDa. Electrophoretic profiles of the samples after digestion revealed small peptides having MWs under 16 kDa. The results demonstrated that the pea flours subjected to various types of fermentation and germination have improved digestibility and thus may have good potential to be used as value-added food ingredients in food applications.

Effect of Processing on Anti-nutrients and In vitro Protein Digestibility of Red Kidney and Navy Bean

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The effects of soaking, steam blanching, cooking on the composition of anti-nutritional factors (total phosphorous, tannins) and in vitro protein digestibility of red kidney and navy bean were investigated. Significant ($p < 0.05$) variations were found among the cooked and raw seed beans with respect to their crude protein, mineral, total phosphorous, and tannin contents. Anti-nutritional factors were significantly reduced ($p < 0.05$) by processing. Cooking was the most effective in reducing total phosphorous and tannin as compared with soaking, and steam blanching. Cooking red kidney and navy bean significantly increased determined protein, whereas ash, and tannin contents were decreased. Red kidney bean contained tannins whereas no tannin was determined in navy bean. Cooking improved protein digestibility of navy bean and red kidney bean. Further, the steam blanched cooked (SBC) navy bean showed greater susceptibility toward enzymatic hydrolysis than the SBC red kidney bean.

Effect of Protein and Glycerol Concentrations on the Mechanical and Water Vapor Barrier Properties of Canola Protein-based Edible Films

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Biodegradable edible films prepared using proteins are both economically and environmentally important to the food packaging industry relative to traditional petroleum-derived synthetic materials. In the present study, the mechanical and water vapor barrier properties of casted canola protein isolate (CPI) edible films were investigated as a function of protein (5% and 7.5%) and glycerol (30%, 35%, 40%, 45%, and 50%) content. Specifically, the tensile strength (TS) and elongation (TE), the elastic modulus (E), the puncture strength (PS) and deformation (PD), and water vapor permeability (WVP) were measured. Results indicated that TS, PS and E decreased, while TE and PD values increased as glycerol concentrations increased for both the 5% and 7.5% CPI films. Furthermore, TS, PS and E values were found to increase at higher protein concentrations within the CPI films, whereas PD values decreased. TE was found to be similar for both CPI protein levels. Water vapor permeability was also found to increase with increasing glycerol and protein content. Overall, findings indicate that CPI films are less brittle, more malleable and allowed for greater water permeability at higher glycerol levels. However, as protein levels increased, CPI films became more brittle, less malleable and also allowed for increased water permeability.

Effect of Plasticizer-type on the Mechanical and Water Vapor Barrier Properties of Canola Protein-based Edible Films Crosslinked With and Without Genipin

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The mechanical and water vapor barrier properties of casted canola protein isolate (CPI; 5%)-based edible films were investigated as a function of plasticizer-type (glycerol, sorbitol and polyethylene glycol 400 (PEG-400; 50%)) in the presence and absence of genipin (1%). The latter is a novel non-toxic fixative that forms intra- and intermolecular covalent bonds between primary amino groups of CPI. Specifically, the tensile strength (TS) and elongation (TE), the

elastic modulus (E), the puncture strength (PS) and deformation (PD), and water vapor permeability (WVP) were measured. Findings indicated that TS, PS and E values for CPI films prepared with sorbitol were the highest, followed by PEG-400 and then glycerol, whereas TE and PD values were greater for films prepared with glycerol, followed by PEG-400 and then sorbitol. In all cases, films prepared with genipin were stronger (greater TS, PS and E) and less malleable (lower TE and PD) than un-crosslinked films. Films also showed greater water permeability when prepared with glycerol, followed by PEG-400 and then sorbitol, however no differences were observed in the presence and absence of genipin. Overall, CPI films prepared with genipin were stronger, less malleable than un-crosslinked films, but had no influence over their water barrier properties.

Processing Proteins From Canola

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Canola non-oil fraction contains ~38% protein. Non-protein components such as fibre, phytates and phenolics reduce value of canola proteins. Canola protein fraction is mainly composed of 11S cruciferin and 2S napin which are different in molecular weight, amino acid composition, and many other properties. Several processes are available to obtain canola protein with minimum contamination of non-protein components. Our objective is to compare these processes for the protein product composition and co-products of the processes. Results of the processes evaluated in the lab and also from literature were used in this study. Protein concentrate (55-75% protein) that can be obtained from FRI-71 process and green biorefinery process contain both 11S and 2S proteins. Protein isolates (>90% protein) prepared by alkali pH extraction and isoelectric precipitation recover 11S with 2S protein, therefore of mixed composition. Protein micellation recovers mostly 11S (or 7S) protein in the micelle and soluble fraction is rich in 2S protein. Sequential extraction similar to Osborne fractionation gives products with mixed composition and, salt and water extraction steps recover most of the recoverable proteins. Process developed by AAFC provides 2S protein rich (~90% protein) and 11S protein rich (65% protein) fractions while a mixed protein containing soluble fibre-rich, seed fibre and soluble sugar fraction can also be obtained.

Formation and Functionality of Napin Protein Isolate-gum Arabic Electrostatic Complexes

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The formation and functionality of napin protein isolate (NPI) - gum Arabic (GA) complexes were investigated as a function of pH at a 1:1 biopolymer mixing ratio (0.1%, w/w) by turbidity and electrophoretic mobility. Coacervation typically follows two pH-induced structure forming events associated with the formation of soluble (pHc) and insoluble (pH(phi)1) complexes, along with the pHs where maximum coacervation (pHopt) and the dissociation of complexes (pH(phi)2) occurs. For NPI-GA, pHc, pH(phi)1, pHopt and pH(phi)2 were found at pHs ~5.6, ~4.6, ~4.1 and ~2.7, respectively. Maximum optical density at pHopt occurred at 1.181, whereas neither NPI nor GA alone displayed any optical density. Net neutrality (zeta potential = 0 mV) shifted from pH 5.0 for NPI alone to pH 2.1 for the NPI-GA mixture. Net neutrality for GA alone was similar to that of the mixed system. The functional attributes (solubility, foaming capacity/stability and emulsion stability) were measured at pH 4.0 for both NPI-GA and NPI alone, corresponding to a pH where insoluble complexes were present, and close to pHopt. Solubility, foam capacity and emulsion stability were similar for NPI and NPI-GA, however foam stability was greatly improved for NPI-GA (78.8%) relative to NPI alone (48.4%).

Effect of pH and Salts on the Physicochemical and Emulsifying Properties of Cruciferin- and Napin-rich Protein Isolates

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The physicochemical and emulsifying properties of a cruciferin- (CPI) and napin-rich (NPI) protein isolate were investigated as a function of pH (3.0, 5.0, 7.0) and NaCl concentration (0, 50, 100 mM). Specifically, surface charge and hydrophobicity, solubility, and emulsifying properties (emulsification activity (EAI) and stability (ESI) indices)

were investigated. In the absence of NaCl, surface charge switched from positive to negative between pH 3.0 and 7.0 for both proteins, with zero net charge occurring at 4.8 and 6.3 for CPI and NPI, respectively. The addition of NaCl, suppressed surface charge on both proteins. Overall, hydrophobicity was much greater for CPI than NPI; declined from pH 3.0 to 7.0; whereas NaCl had little effect. Overall, NPI solubility was greater than CPI; however decrease and increase as pH and NaCl levels increased, respectively. Overall, EAI and ESI values were slightly greater for NPI than CPI; increased and decreased as pH was reduced from 7.0 to 3.0, respectively; and the addition of NaCl had a negative effect on both EAI and ESI. In summary, NPI is less hydrophobic, less charged, more soluble, and has better emulsifying properties than CPI. All properties were significantly affected by changes in pH and the presence of NaCl.

Usage of protein enrichers to improve the rheological characteristics of unleavened dough

O. Shanina⁽¹⁾, K. Dugina⁽²⁾, A. Teymurova⁽³⁾, V. Zverev⁽⁴⁾

⁽¹⁾Petro Vasilenko Kharkiv National Technical University of Agriculture, Ukraine ⁽²⁾Petro Vasilenko Kharkiv National Technical University of Agriculture, Ukraine ⁽³⁾University of Saskatchewan, Canada ⁽⁴⁾Petro Vasilenko Kharkiv National Technical University of Agriculture, Ukraine One of the effective ways to increase nutritional value of cereals is combining them for mutual enrichment. Using computational methods, we were able to develop an optimized flour mixture composition from different types of grain with enhanced nutritional value. Glycemic indexes of these compositions were reduced to 45% compared to the wheat flour (70%) and protein quality indexes were increased in 3-5 times. However, the main problem of products based on flour mixtures is to provide the traditional structure to the consumers. It is important to note that variety of ingredients that differs by origin, functionality and nutritional properties has successfully been applied. Currently, functional animal proteins produced from recycled raw meat materials and transglutaminase enzymes are considered the most effective, safest and eco-friendly ingredients. These additives significantly improved the properties of dough and the quality of the final product. An increase of dough shear stress with 47-65% was observed in the presence of these additives. The irreversible relative strain was reduced with 44-53%; viscous-elastic properties were improved. The appearance and consistency of these products improved. This study strongly indicates importance of protein additives both as structure forming agents and protein enrichment substances of unleavened dough.

Animal proteins and enzymes - effective dough and bread improvers

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In general, bread is considered as a traditional food consumed daily, therefore, production of high quality is at great demand. Consumers expect products with high nutritional value in terms of protein (quantity/quality) and excellent organoleptic properties (texture). Currently, the supplementation of bread with certain proteins is contradictory regarding their effect, because although it results in an increase of protein quantity and quality in bread it may degrade the bread structure. However, improvement of the structure with chemical additives would negatively influence the nutritional value of bread. In our view, by-products of peas, beans, chickpeas or potatoes can be used as effective and safe protein enrichers. Protein concentrates obtained from collagen raw materials and enzymes (e.g., transglutaminase) are considered as the most perspective bakery improvers, which can strengthen the weak structure of bread dough. Protein-protein interactions between the proteins of raw materials and additives are expected. This hypothesis was confirmed by the infrared spectroscopic and titration analyses. We observed that in the presence of additives wheat flour proteins aggregated within shorter period of time and at higher optical density in comparison to the control.

Bile acid-binding properties of spent hen protein hydrolysates

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The poultry egg industry generates enormous amounts of spent laying hens after the laying cycle, and the byproducts contain proteins that are currently underutilized in the food system. The objective of this work was to investigate the potential use of spent hen proteins as precursors of bioactive peptides. Direct hydrolysis of ground spent hen breast

meat with three different enzymes, Alcalase®, bromelain and pancreatin resulted in three hydrolysates with yields of 26%, 19% and 23% (dry weight basis), respectively. The hydrolysates were then evaluated for bile acid-binding activity using sodium cholate (SC) and sodium taurocholate (STC). The ability of peptides to bind bile acids can enhance their hypocholesterolemic activity because of potential alteration of enterohepatic bile acid circulation, which can concomitantly increase hepatic cholesterol metabolism. In vitro evaluation indicated that the hydrolysates, at 1 mg/mL, exhibited considerable activities of 13-32% and 24-31% in binding SC and STC, respectively compared to equal concentration of synthetic resin, cholestyramine, a bile acid sequestrant that showed 77% and 81% binding of SC and STC, respectively. The hydrolysates derived from alcalase and bromelain hydrolysis had the best activity in binding the bile acids. High molecular weight peptide aggregates were also prepared from the respective hydrolysates but these derivatives did not result in enhanced bile acid-binding activity. These results indicate that enzymatic hydrolysis of protein-rich spent hen meat with Alcalase® and bromelain can result in natural peptide products with prospective application in the management of hyperlipidemia and associated cardiovascular disease.

Functional properties of hemp seed albumin and globulin protein fractions.

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Hemp seed storage proteins, the albumin and globulin were isolated by water and salt extraction, respectively. Functional properties such as protein solubility, gelation, water and oil absorption capacity, foaming and emulsification were investigated in relation to the effects of pH, ionic strength, and protein concentrations. The extracted globulin had 100% protein content while albumin had 81% protein content; however the protein and gross weight yields were higher for albumin than globulin. Non-reducing SDS-PAGE analysis showed that the globulin fraction had three major polypeptides (7, 11 and 266 kDa) while the albumin fraction had six major polypeptides (19-242 kDa). The albumin and globulin had minimum solubility at pH 3 and 5, respectively, but both protein fractions showed maximum solubility at pH 9. The albumin had significantly higher ($p < 0.05$) foaming capacity but lower foam stability when compared to globulin. Emulsion capacity of both fractions was directly related to their solubility and surface characteristics. Their combinational ratios (albumin:globulin) gave significant ($p < 0.05$) improvements in oil absorption capacity, foaming capacity and emulsifying properties above values obtained for individual fractions. We concluded that hemp seed albumin and globulin fractions had functional properties that could provide alternative protein ingredient sources for the formulation of novel food products.

CANCELLED-Biodegradable Plastics from Proteineous Meals via Thermoplastic Extrusion Method

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CANCELLED-New Value-Added Engineered Biomaterials from Soy Meal

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