PROTEIN AND CO-PRODUCTS

PCP 1b: Protein Biofunctions

Chairs: Kaustav Majumder, University of Nebraska, USA; Hisham Ibrahim, Kagoshima University, Japan; and Hitomi Kumagai, Dept. of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, Japan

Wheat-Gliadin Allergy Induced by Cutaneous Sensitization Yusuke Yamaguchi*[1], Narumi Matsukaze[1], Ryosuke Abe[1], Hitoshi Kumagai[2], and Hitomi Kumagai[3],[1]*Nihon University, Japan;* [2]*Faculty of Home Economics, Kyoritsu Women's University;* [3]*Dept. of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, Japan*

Food allergy was previously considered to be caused by intestinal sensitization. However, another type of food allergy induced by cutaneous sensitization has recently been reported. HCl-treated wheat protein (HWP) used in facial soap to enhance foaming properties of the product was found to cause allergic reaction when wheat products were ingested after the use of this soap, but the mechanism of the allergic sensitization has not been fully elucidated. As HCl treatment causes not only protein hydrolysis but also protein deamidation, these two factors are possible reasons for this immediate hypersensitivity. In this study, we examined the effect of hydrolysis and deamidation of gliadin on allergic sensitization. Gliadin, a major wheat allergen, was extracted from gluten using 60% ethanol and then hydrolyzed and deamidated by HCl to give hydrolyzed and deamidated gliadin (HDG). Hydrolyzed gliadin (HG) was prepared from gliadin using pepsin. Deamidated gliadin (DG) was prepared by treatment of gliadin with cation-exchange resins which deamidate the side chain of amide groups without causing peptide-bond hydrolysis. Each gliadin sample was applied onto mouse skin for a certain period of time. Then, the same gliadin sample used for cutaneous sensitization was intraperitoneally injected, followed by collection of blood, skin and small intestine to measure allergic markers. Severe allergic responses were observed only in the HDG group after intraperitoneal injection of HDG, while the DG and HG groups showed low or no allergic reactions. These results indicate that both hydrolysis and deamidation of gliadin are important to cause cutaneous sensitization for gliadin.

Anti-viral and Anti-allergic Activities of Highly Phosphorylated Casein Phosphopeptide Shigeru Katayama*, and Soichiro Nakamura,*Shinshu University, Japan*

Casein phosphopeptide (CPP) is a tryptic digest of bovine caseins and have multiple bioactive functionalities, including improved calcium absorption and immunomodulatory activity. In this study, we investigated the anti-viral and anti-allergic activities of CPP-III, mainly consisting of bovine α_{s2}-casein (1-32) and β-casein (1-28). The effects of additional phosphorylation and dephosphorylation on their activities were also assessed to clarify the role of phosphate groups. CPP-III was kindly provided by Meiji Food Materia Co., Ltd. (Tokyo, Japan). Additionally phosphorylated CPP-III (P-CPP-III) was prepared by dry-heating in the presence of sodium pyrophosphate, while dephosphorylated CPP-III (D-CPP-III) was obtained by enzymatic treatment using alkaline phosphatase. Feline calicivirus (FCV) strain F9, a typical Norovirus surrogate, and CRFK cells were used as the target virus and its host cells, respectively. FCV infection decreased the viability of host cells; however, the treatment with P-CPP-III attenuated the cell death, and type 1 interferon expression was upregulated

compared to that treated with native CPP-III. In contrast, dephosphorylation of CPP-III resulted in a decrease in the anti-FCV effect. In OVA-sensitized mice as a mouse model of egg allergy, oral administration with native CPP-III and P-CPP-III for 42 days developed less hypersensitivity; in particular, P-CPP-III-fed mice exhibited a significant reduction. Marked increase in the differentiation of regulatory T (Treg) cells was observed in the P-CPP-III treatment, while D-CPP-III showed similar levels compared to sham-treated mice. These results suggest that the anti-viral and anti-allergic activities of CPP were enhanced by the introduction of additional phosphates and conversely weakened by their elimination.

Molecular Properties of Food Allergen Proteins

Philip E. Johnson*, *University of Nebraska-Lincoln, USA*

Food allergy is caused by specific proteins within allergenic foods. Despite the identification of many such allergenic proteins, a simple molecular description of what makes one protein allergenic while others are not, remains elusive. An understanding of chemical or structural aspects of food proteins which makes them prone to sensitizing individuals and to eliciting responses is crucial to our future ability to identify potential allergens in novel foods, reduce allergenicity in existing foods, and treat food allergic individuals. This presentation will summarize current understanding of the molecular basis of food allergy, drawing on original research from our group. We will discuss the types of proteins that are commonly allergenic, their functions, chemistry and structure. How these proteins interact with the immune system via the digestive tract will be described, as will the current state of knowledge as to how commonly-used food processing techniques may affect allergenicity. We will address how this knowledge is applied to current regulatory frameworks with respect to novel and existing foods.

Effect of whey peptides on metabolism and insulin signaling in muscle and fat cells

Kenneth D'Souza*[1], Angella Mercer[1], Hannah Mawhinney[2], Thomas Pulinilkunnil[1], Chibuike C. Udenigwe[2], and Petra Kienesberger[1], *[1]Dalhousie Medicine New Brunswick, Canada; [2]University of Ottawa, Canada*

RATIONALE: Adipose tissue and skeletal muscle dysfunction are hallmarks of obesity and insulin resistance. Bioactive peptides derived from food sources including milk and dairy products have gained interest for their roles in obesity and insulin resistance. However, it remains unclear whether and how whey impacts adipose and muscle metabolism and insulin function. HYPOTHESIS: Bioactive whey peptides have an insulin-sensitizing effect on adipocytes and muscle cells. METHODS: Whey peptide mixture was generated via the hydrolysis of whey protein with pepsin and pancreatin. 3T3-L1 pre-adipocytes were incubated with 2.5 mg/ml bovine serum albumin (BSA) or whey peptides during differentiation. Lipid accumulation and the expression of proteins involved in lipid metabolism was analyzed. Insulin resistant C2C12 myotubes were incubated with BSA or whey peptides for 16 h followed by insulin signaling analysis. RESULTS: In 3T3-L1 cells, whey peptides increased expression of the master-regulators of adipogenesis, C/EBPα and PPARγ. This was associated with upregulation of perilipin and adiponectin, markers of enhanced lipid storage and insulin sensitivity, and increased triacylglycerol accumulation. In C2C12 myotubes, whey peptides protected from palmitate-induced insulin resistance, as determined by improved AKT phosphorylation and Glut4 expression. Insulin sensitization of C2C12 myotubes was accompanied by decreased inflammation and ER stress following whey peptide treatment. CONCLUSIONS: Whey

peptides promote differentiation and PPARγ activation in adipocytes. In myotubes exposed to an obese-diabetic milieu, whey peptides ameliorate insulin resistance, potentially by reducing inflammation and ER stress. Taken together, our data suggest that whey peptides directly enhance lipid metabolism and insulin function in adipocytes and muscle cells.

Glucagon-like peptide-1 is released from the distal small intestine by a standard diet containing casein as a protein source but not by a non-protein diet in rats Tohru Hira*, Madoka Sekishita, and Hiroshi Hara, *Hokkaido University, Japan*

Objective It has been considered that glucagon like peptide-1 (GLP-1) is postprandially released from the distal small intestine due to observations that GLP-1-producing enteroendocrine cells mainly located in the region. Although it is well known that macronutrients (carbohydrate, fat, and protein) respectively stimulate GLP-1 secretion, roles of each nutrient in "meal"-induced GLP-1 secretion remain unclear. The present study was conducted to examine site-specific GLP-1 secretion under a physiological (conscious and unrestrained) condition, and the role of a dietary protein in postprandial GLP-1 response. Methods Male Sprague Dawley rats were equipped with a catheter inserted into either the portal vein or the ileal mesenteric vein. After recovery, rats were given test diets with or without a protein (casein). Blood samples were collected from the portal vein or the ileal mesenteric vein through the catheter, before and after the voluntary ingestion of test diets. Results Postprandial glucose concentrations were higher in the portal vein than the ileal mesenteric vein. After consuming a standard diet containing casein as a protein source, GLP-1 concentration was higher in the ileal mesenteric vein than the portal vein. In contrast, a protein-free diet did not increase GLP-1 concentration both in the portal vein and the ileal mesenteric vein. Conclusions These results suggest that after a meal ingestion, GLP-1 is immediately released from the distal intestine under physiological conditions. Although the potency possibly depends on varieties and forms of protein sources, a protein source such as casein in the diet seems to have an essential role in postprandial GLP-1 secretion.

PCP 2a: Emerging Sources of Protein

Chairs: Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada; and Andrea Liceaga, Purdue University, USA

Value-added Applications of Spent Hen as Nutraceuticals and Functional Food Ingredients Hongbing Fan*, and Jianping Wu, *University of Alberta, Canada*

Spent hens are the birds which reach the end of their laying cycles and are a major byproduct of the egg industry. There are more than 30 million spent hens produced every year in Canada. They are partly processed for food and feed uses which are of limited economic value to the industry; a majority of them are disposed by burial, composting, and incineration, which negatively affect environment due to associated carbon footprints as well as bring animal welfare issues. Recently, new processing techniques have been used to enlarge value-added applications for spent hen, since it is rich in various proteins including muscle proteins and collagens, though considered as a byproduct. The muscle proteins have been used to generate bioactive peptides including with immunomodulatory, anti-inflammatory, and antihypertensive functions. While, the collagens including from skin, bone, and connective tissues can be used to produce low-molecular-weight peptides with various beneficial effects such as wound healing, skin and bone health, among others. These recent progresses indicate possible nutraceutical and functional food applications for spent hen, which will not only bring in extra revenue for egg farmers but also further strengthen the interaction between the egg industry and health care research.

Recovering Amino Acids and Peptides in an Integrated Algal Biomass Refinery Tao Dong*, Nick Nagle, Eric P. Knoshaug, Philip Pienkos, and Lieve Laurens, *National Renewable Energy Laboratory, USA*

Though microalgal biomass offer a greater theoretical biofuel yield over terrestrial-based feedstocks, reducing the costs of algal biofuel remains a challenge for commercialization. Our strategy is to develop value-added co-products in tandem with the production of commodity biofuels, and we have developed the Combined Algal Processing (CAP) biorefinery process for this purpose. In this presentation we will describe one configuration of the CAP process in which algal biomass was hydrolyzed by dilute acid at elevated temperature to rupture cell walls and release monomeric sugars. The hydrolyzed algal slurry was used for fermentation to convert fermentable sugars into ethanol, and the lipid was recovered in a subsequent lipid extraction, leaving protein rich residues. The soluble fraction of the residue, named CAP liquor, was used as a source of amino acids (AAs) and peptides to expand algal co-products portfolio (e.g. non-isocyanate polyurethanes), with an aim to further reduce algal biofuel cost. Several resins were tested in terms of recovering of AAs and peptides. Up to 65% of total AAs and peptides were recovered and the purity of the obtained product reached 90%, which was a 4.75-fold increase from the CAP liquor.

Improvement of Functional and Bioactive Properties with Microwave-Assisted Hydrolysis of Chia Seed (Salvia hispanica) Protein Uriel C. Urbizo*, M. Fernanda San Martín-Gonzalez, Jose G. Bravo, and Andrea M. Liceaga, *Purdue University, USA*

Over the past few years, chia seed (Salvia hispanica) (CS) has gained popularity worldwide. Unfortunately, mucilage surrounding the seed limits the applicability of the available protein. The implementation of new technologies can aid the protein extraction and improve their functional and bioactive

properties. Ultrasonication was used to successfully separate mucilage (7.8% yield) from the protein. Proteins were enzymatically hydrolyzed comparing conventional (WB) and microwave-assisted (MW) treatments. Hydrolysis included single enzyme Alcalase (A) or sequential digestion with Alcalase-Flavourzyme (AF). Overall, CS protein hydrolysates (CSPH) generated with MW, and sequential treatment (AF-MW) showed significantly higher (pAF-MW>A-MW>A-WB>control. No difference (p

Introducing Hairless Canaryseeds: An Emerging Source of High Quality Protein Emily Mason*[1], and Lamia L'Hocine[2], *[1]McGill University, Canada; [2]Agriculture and Agri-Food Canada, Canada*

Hairless canaryseeds were recently approved for human consumption in Canada and the United States. Previously, the seeds were only used as birdseed because they were lined with fine, hair-like, carcinogenic silica fibers. However, through mutagenesis and breading techniques, a new hairless variety has been developed which has been deemed safe for human consumption. This true cereal grain is exceptionally high in protein (23%) as compared to other cereals in the same family. In regard to its protein quality, canaryseed has a well balanced amino acid profile and is particularly high in tryptophan, an essential amino acid normally lacking in cereals. Bioactive peptides from canaryseed proteins have demonstrated antioxidant, antihypertensive, and antidiabetic activity to date. In addition, the seeds possess unsaturated fatty acids, minerals, and phytochemicals which further contribute to their health promoting properties. Furthermore, the seeds are gluten-free and could replace gluten containing cereals in some applications to create new food products for gluten sensitive individuals. Because of their properties, canaryseeds can be regarded as a functional food or ingredient and used in food products to improve their overall nutritional value, and provide desirable health promoting effects.

Effect of Enzymatic Hydrolysis and Microwave Energy on Allergenicity of Edible Cricket (*Gryllodes sigillatus*) Protein Hydrolysates Andrea M. Liceaga[1], Philip E. Johnson[2], and Felicia G. Hall*[1], *[1]Purdue University, USA; [2]University of Nebraska-Lincoln, USA*

Emerging protein sources, such as crickets, have gained attention in the North America and Europe. However, evidence has confirmed that cricket protein can cross-react with common crustacean allergens. Enzymatic hydrolysis and microwave treatments are being considered as approaches to produce hypoallergenic peptides for food applications. In our work, we observed positive tropomyosin reactivity from cricket protein to IgE from shrimp-allergic patients. Allergenic responses were modified after enzymatically hydrolyzing the protein to varying degrees of hydrolysis (DH). Tropomyosin-IgE binding decreased as DH values increased with almost no response in samples >60% hydrolyzed. Unexpectedly, tropomyosin-IgE reactivity was higher in samples with 50% DH than those with 10-40% or >50% DH. To examine this further, cricket protein were hydrolyzed using microwave processing to obtain 50% DH. Microwave-treated samples exhibited a decreased IgG-tropomyosin response (4.6 ng/mL) compared to non-microwave treated hydrolysates (51.4 ng/mL). Further, Raman spectroscopy was used to elucidate tropomyosin structural changes induced by the different treatment conditions. Pure tropomyosin, tropomyosin extracted from shrimp and tropomyosin extracted from whole crickets, all showed distinct peaks at 1647 nm (amide I), 1097 nm (C-H stretching), 782 nm (associated with the microenvironment of Trp), and 552 nm (S-S trans-gauche-trans region).

Shift in Raman bands, decreased peak intensity or absence of these bands were observed in the microwave-treated cricket protein, correlating to the decreased IgG reactivity Enzymatic hydrolysis coupled with microwave treatment may effectively induce conformational changes in the epitope region of the tropomyosin, allowing access for the proteolytic enzyme to cleave the target sites.

Optimization of Process for the Production of a Light-coloured and Highly Soluble Sunflower Protein Isolate Sara Albe Slabi*[1], Christelle Mathé[2], Melody Basselin[3], Xavier Framboisier[4], Arnaud Aymes[4], Olivier Galet[5], and Romain Kapel[4], *[1]Reaction and Process Engineering Laboratory UMR-7274, Avril Group, France; [2]Reaction and Process Engineering Laboratory, France; [3]LRGP - UMR 7274, France; [4]Reaction and Process Engineering Laboratory UMR-7274, France; [5]Avril Group, France*

The growing world population sets a new challenge for food industry. Sunflower proteins (30-50 %/dm of meal) are considered very promising for human nutrition thanks to attractive nutritional and functional properties. However, during solid/liquid extraction phenol-protein interactions induce an unsuitable green colour. Furthermore, conventional protein purification by acidic precipitation leads to poor solubility of protein products. Therefore, the main objective of this work was to propose an optimized process for protein extraction and purification by ultrafiltration that yields in light-coloured and highly soluble isolate. First, the influence of pH (6-9) and NaCl concertation (0-0.5 mol.L-1) on protein extractability and phenolic contamination was investigated. Then, a multi-objective optimization with constraints was performed. Finally, the best scenario for ultrafiltration was established by determining retention coefficients of target compounds and modelling a protein purity. As a result, the developed models revealed a positive, synergic impact of pH and ionic strength on protein extractability. Irreversible phenol-protein interactions dramatically increased with extraction pH. Interestingly, a protective effect of NaCl was also shown. Based on the results of multi-objectives optimization the extraction at pH 7.0/0.5 mol·L-1 NaCl was the best trade-off between all competing criteria. The extracted proteins were subsequently purified by ultrafiltration using an original protocol with saline diafiltration. The developed process resulted in production of light-coloured and highly purified proteins (99.9 %/dm). Additionally, the solubility of protein isolate (≥75 % at pH 7) was considerably improved. Thus, the proposed alternative process could be an answer for sustainable valorization of sunflower meal in food industry.

EAT 2a/PCP 2b: Plant Protein Utilization in Food Products
Chairs: Graciela Padua, University of Illinois, USA; and Baraem Ismail, University of Minnesota, USA

Rheological Assessment of Ethanol Induced Plant Protein Gels Nahla Kreidly*[1], Graciela W. Padua[2], and Hakime Yavuz[1], *[1]University of Illinois at Urbana Champaign, USA; [2]University of Illinois, USA*

Plant protein based gels are currently of high interest due to consumers' preference over the use of animal proteins. Protein gelation is often achieved by heat treatment or by the addition of gelling agents. However, heat treatment may damage nutrients, while the addition of gel inducing agents may be objectionable in certain foods. In this study, we further investigate the effect of a novel ethanol induced gelation process that is carried out at room temperature. It is based on the instantaneous gelling properties of certain proteins when coming in contact with ethanol. Dynamic rheological measurements using oscillation tests were employed to investigate the viscoelastic properties of ethanol induced gels from almond, lentil, and pea proteins. Rheological assessment also aimed to reveal the effect of protein content (10-20% w/v) and ethanol (30-80% v/v) on the gelling ability of plant proteins in binary solvent systems of ethanol-water. All proteins formed instantons gels with G' > G'' immediately upon contact with ethanol. The stiffest gels were observed at a 40% ethanol and 20% protein content for all proteins. Structural differences between untreated protein powders and protein gels were investigated by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Protein gels developed a porous structure after drying at room temperature, in contrast with the granular structure untreated protein powders. This work aims to enhance the utilization of plant proteins in the food industry as functional and inexpensive ingredients, by studying their ability to form instantaneous gels using novel ethanol induced gelation.

Plants to Meat: Utilizing Plant Proteins to Satisfy the Carnivores Ines Resano Goizueta*, *Impossible Foods, USA*

Animals to make meat is a prehistoric and unsustainable technology. Animal agriculture occupies almost half the land on earth, consumes a quarter of our freshwater and destroys our ecosystems. Due to the impact of animal farming on our world, there is commercial interest in developing foods made from plants that satisfy the carnivore to remove the farm-animal from the human diet. Plant-based meat companies are deconstructing the eating experience and re-designing meats from scratch by using only ingredients from plants. Consumers eat meat not only because it is delicious but because it is a good source of protein. Plant proteins replacing animal proteins have to provide the same protein quality and the same sensorial experience. This requires the proteins to be functional and flavorless. At the same time, the protein must be scalable and affordable. There is enough plant protein grown in the world to substitute animal protein but not all that protein is high quality. Protein functionality allows us to make a dynamic product where the proteins can gel, emulsify and stabilize the food during handling and cooking, but many current protein isolation processes limit the functionality. Proteins must be low in flavor and not distract from the meat-flavor, but bitterness and off flavors are present in many commercially available proteins. Developing a supply of plant proteins that are functional and have low flavor, at a scale that allows us to feed the world is one of the biggest challenges we face in the 21st century.

Soy Protein-based Nanoparticles and Derivatives as Bioavailability Enhancers for Bioactive Compounds Qin Wang*, *University of Maryland, USA*

Soy protein is one of the most widely utilized plant proteins with unique nutritional values. This study systematically investigated

the preparation, characterization, and application of soy protein-based nanoparticles as effective nutraceutical/drug carriers. The particle formation involved partial unfolding of protein molecules, limited aggregation in the presence of the antisolvent, hardening of particle structure via chemical bonds, and refolding of the protein molecules within the particles. Satisfactory dispersion stability, encapsulation efficiency (EE), and time-dependent release of curcumin, a chemopreventive compound, were observed. The nanoparticles were further subjected to conjugation with folic acid, a cancer cell-targeting ligand. The conjugation led to more extensive particle formation and higher loading efficiency (LE), probably due to an increase in surface hydrophobicity. More importantly, a pronounced increase in the accumulation in tumor cells such as Caco-2 was achieved upon folic acid, which demonstrated the potential of this technique for the targeted delivery of anti-cancer drugs. To overcome the rapid digestion of soy protein nanoparticles in the gastrointestinal tract, carboxymethyl chitosan (CMCS) was employed as a second coating layer by a simple ionic gelation method. CMCS rendered the gelation of soy protein molecules more effectively and required lower concentration of calcium crosslinkers. The CMCS/soy protein complex nanoparticles exhibited satisfactory EE for vitamin D3, reduced release in simulated gastric environment, and enhanced release under simulated intestinal conditions. This study demonstrated the potential of soy protein-based nanoparticles as an effective vehicle for carrying and delivering bioactives for various applications.

Overcoming the Challenges in the Production and Utilization of Plant Protein Isolates in Food Products Nagul Naguleswaran*, *Ingredion, USA*

The utilization of plant proteins in food products has rapidly increased during recent years. The food industry prefers the use of plant protein isolates primarily due to their high protein content and high nutritional value. However, there are numerous challenges in formulating food products with plant protein isolates, particularly in achieving the target functional properties in the ingredients. Although there is a number of plant protein isolates currently available in the market, many of them could not be used as alternatives to animal proteins. Several factors influence the production of plant protein isolates with desired functional properties that match the functionalities of animal proteins. At present, the protein isolates are produced from oilseeds, pulses, and cereals. The physicochemical and functional properties of the plant protein isolates vary primarily due to both the source and processing methods. This presentation reviews the challenges and opportunities associated with the production of selected plant protein isolates, with desired functional properties.

Improvement of Targeted Pea Protein Functionalities for Beverage Applications Serpil Metin*[1], Sonia Han[2], and Tasha Hermes[2],[1]*Cargill R&D, USA;* [2]*Cargill, USA*

There is a strong interest in the consumption of beverages containing plant proteins due to potential health benefits. Pea protein is an attractive protein for some consumers, especially for vegetarians, vegans and flexitarians. However, utilization of pea protein in beverages have challenges due to its low solubility, sedimentation, flavor, and thermal stability. Pea protein can be modified for target functionalities desired for beverage applications. In general, food protein functionality is commonly measured from the perspective of single protein molecules in solution. While this approach is very beneficial in understanding of functionalities of proteins, it is somewhat limited in predicting performance in final food applications. This presentation will include modification of pea protein to improve its solubility and flavor for beverage applications as well as measurement of target functionalities of both intact and modified proteins. It will also include an approach taken for prediction of performance

of both proteins in the presence of other ingredients (e.g. sweeteners, stabilizers, flavoring agents, etc.) and during processing steps in a beverage application.

Structural and Functional Properties of Plant Protein Isolates and Hydrolysates for Various Applications Baraem Ismail*, *University of Minnesota, USA*

The demonstration of equivalent or superior/new functionality of novel plant proteins compared to existing alternatives is essential to both the food industry and the consumer. However, there is limited consumer and producer knowledge of plant proteins other than soy. Food producers are seeking information on the nutritional, physiological and functional characteristics of plant proteins. Additionally, efficient extraction and functionalization procedures are needed. This presentation will cover the evaluation of various plant proteins from pea, camelina, and pennycress, highlighting their structural and functional properties as impacted by extraction and functionalization procedures, as well as potential applications. Structural characteristics and functional properties of the protein concentrates, isolates and hydrolysates will be discussed and compared to reference proteins, whey protein isolate (WPI) and soy protein isolate (SPI).

PCP 3a: Proteins in Delivery Functions

Chairs: Chibuike Udenigwe, University of Ottawa, Canada; and Lingyun Chen, University of Alberta, Canada

Beta-lactoglobulin and its Cationic Derivatives for Effective Encapsulation and Delivery of Bioactives Qin Wang*, *University of Maryland, USA*

β-lactoglobulin (BLG) is the major component of whey protein and a natural carrier for various lipophilic nutrients. In this work, BLG nanoparticles with desirable loading efficiency (LE) and low crosslinker concentration were synthesized. Using curcumin as a model drug, we demonstrated that the LE could be improved by up to 157% by maintaining low antisolvent content before mild evaporation. Moreover, the optimal level of glutaraldehyde crosslinker decreased by 50% as the curcumin/protein ratio increased, suggesting that toxic cross-linkers could be partly replaced with natural phenols such as curcumin. We further decorated BLG with various cationic moieties with different surface charge and hydrophobicity. The cationization procedure resulted in a higher percentage of random coils and lower content of β-sheets. In addition, the cationic corona conferred CBLG with superior integrity and drug retention under gastrointestinal conditions, at most 40-fold higher mucoadhesion determined by quartz crystal microbalance (QCM), up to 30-fold greater transepithelial permeation and, at most 285% higher cellular uptake, compared to BLG. Furthermore, the more hydrophilic CBLG species exhibited better mucoadhesion, while the more hydrophobic one exhibited higher cellular uptake. When comparing the protein molecules and nanoparticles, the former more cytotoxic and exhibited up to 175% higher tight junction-opening capacity, whereas the latter displayed up to 770% higher mucoadhesion, greater transepithelial permeation and elevated cellular uptake. This study demonstrated the great potential of BLG in encapsulating and delivering functional ingredients. It also sheds some light on the development of protein-based nanoencapsulants and their performance upon oral administration.

Plant Protein Based Nano-emulsions for Delivery of Vitamin D Lingyun Chen, Zhigang Tian, and Niharika Walia*, *University of Alberta, Canada*

Objective: In North America, a significant population is vitamin D deficient due to limited sunlight exposure. Increasing research has shown that vitamin D apart from its skeletal functions, also has potential to lower the risk of chronic diseases. However, the most challenging barrier has been poor bioavailability of vitamin D due to its low aqueous solubility. Methods used: In this work, pea protein nano-emulsions were developed as a delivery system to encapsulate vitamin D with the aim to increase its stability and bioavailability. Results: The nano-emulsions exhibited controllable sizes (170- 350 nm), good stability with zeta-potential value of around -25mV and high encapsulation efficiency (94-96%). Vitamin D encapsulated in an emulsion system showed better oxidation stability as compared to free vitamin D suspension. Using Caco-2 cell model, the nano-emulsions were found relatively safe after 6 hrs of incubation with cell viability values >80%. Cellular uptake efficiency of small sized nano-emulsions was found ~ 2.5 folds higher (p

Prolamin-based Nanoparticles as Sustained Release Drug Delivery System Yue Zhang*, *University of Nebraska-Lincoln, USA*

Many plant-based bioactive compounds such as sulforaphane show limited shelf-life due to their high reactivity, while their clinical applications may also be restricted by the low gastrointestinal absorption and poor

bioavailability, which may be overcome by biodegradable controlled-release delivery system. Prolamin-based delivery system is of special interest due to their abundance, low cost and unique interfacial properties. In the present study, two prolamins from cereal grain (maize and proso millet) were extracted and employed to develop nanoparticles to protect and release bioactive compounds such as sulforaphane in a controlled manner. Prolamins from maize (CP) and proso millet (MP) were isolated by 70% (v/v) ethanol. To prepare nanoparticles, two g protein isolate was dissolved in 100 mL of 80% v/v aqueous ethanol at 60 oC. Sulforaphane was added to protein dispersions at a mass ratio of 1:100 after cooling. The mixtures were stabilized by 0.05-0.5% w/v sodium caseinate. Ethanol was removed and nanodispersions were freeze-dried. The stability and morphology of nanoparticles in the absence/presence of sulforaphane were respectively characterized. CP nanoparticles showed smaller particle size and higher stability than MP nanoparticles but similar encapsulation efficiency of sulforaphane (89.5% vs 88.1%). MP showed a more amorphous structure than CP nanoparticles as observed in SEM. Compared with CP nanoparticles, sulforaphane encapsualted in MP nanoparticles presented a slower release rate using an in vitro gastrointestinal model. The findings in this study are useful for fabricating, optimizing and selecting prolamin-based delivery systems.

Development of Pickering Oil-in-Water Emulsions Stabilized by Desolvated Pea Protein Nanoparticles Chi Diem Doan*, and Supratim Ghosh, *University of Saskatchewan, Canada*

The present work demonstrates the capacity of pea protein nanoparticles (PPP) in the development of oil-in-water emulsions in comparison with soluble pea proteins (PS) at pH ranging from 6.0 to 9.0. PPP was synthesized by ethanol desolvation where a 3% pea protein solution was two-times diluted in anhydrous ethanol at pH 10 under constant stirring at room temperature. After desolvation, the PPP were recovered by centrifugation followed by re-dispersion in water at various pH values and homogenization at 5,000 psi to control particle size. 5% canola oil-in-water emulsions were then prepared with 2% PPP using high-pressure homogenization. The emulsions showed monomodal droplet size distribution with an average size between 200-300 nm, significantly lower than that obtained using pea protein solution. The zeta potential of the PPP-emulsions was also significantly higher than the PS-emulsions. Confocal microscopy showed the presence of PPP at the surface of oil droplets. The higher stability of PPP-emulsions compared to PS-emulsions was demonstrated by much lower flocculation, coalescence, and creaming indices during a 3-month storage at room temperature and also during freeze/thaw cycles. Despite PS-emulsions possessing better stability against heating at 95°C, heated PPP-emulsions still displayed a monomodal droplet size distribution. Higher stability of Pickering emulsions was explained by the formation of a stronger interface, higher surface activity and higher adsorbed protein% for the PPP compared to the PS.

PCP 3b: Biotransformation of Proteins

Chairs: Buddhi Lamsal, Iowa State University, USA; and Xiaonan Sui, Northeast Agricultural University, China

Recent Progress in Preparing Bioactive Peptides from Food Proteins Jianping Wu*, *University of Alberta, Canada*

The presence of encrypted bioactive peptides in food proteins has attracted substantial interest from the academic and the industry in developing technologies in releasing these sequences from their parent proteins. Proteolysis and fermentation are two most sought-after approaches in preparing bioactive peptides; other techniques are also applied to prepare bioactive but are often associated with lack of specificities. Despite the fact that an increasing number of new peptides are continuously reported in literature, the rationale behind the selection of the protease pertinent to specific protein substrate has not been well justified. The current approach to bioactive peptide discovery was built upon the concept of investigating one protein and one enzyme at a time and has long since reached its throughput limits. The conventional peptide discovery strategy generally requires multi-step purifications (which often take months) to generate a new sequence, which is labor-intensive and costly. Moreover, the conventional activity-based purification steps might exclude potent but minor peptides in the hydrolysate. The purpose of this presentation is to discuss new strategies in discovering novel bioactive peptides. Recent developments in bioinformatics and analytical tools (proteomics) shed new light in the area. Integration of bioinformatics and quantitative structure activity relationship, along with in silico digestion, has made it a reality of discovering new peptides through sequence-based approach; examples for discovery of new peptides will be discussed.

Fabrication and Characterization of Gelatin-Based Nanofibers by Emulsion Electrospinning Cen Zhang*, and Hui Zhang, *Zhejiang University, China*

The aim of the work was to fabricate gelatin-based fibers by electrospinning of corn oil-in-water (O/W) emulsions, which were expected to enhance the encapsulation efficiency, stability, and bioavailability of bioactive compounds, as well as achieve targeted delivery and controlled release. The morphology of electrospun nanofibers was analyzed by scanning electron microscopy (SEM), transmission electron microscope (TEM), and confocal laser scanning microscopy (CLSM). The thermal behavior and conformational changes of nanofiber mats were evaluated using thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR). Additionally, the rheology and microstructure of the gelatin-stabilized emulsions were characterized by rheological measurements and optical microscopy. Results showed that the emulsions showed shear-thinning and predominantly elastic gel behaviors. Compared with the uniform nanofibers by gelatin electrospinning, the beads-in-string fiber structures by gelatin-stabilized emulsion electrospinning were observed in SEM images. CLSM and TEM observations confirmed that the encapsulated oil was randomly distributed as core, especially inside the beads. The electrospun fibers showed good storage stability and thermal decomposition stability. Furthermore, an oppositely charged polysaccharide was added to the primary gelatin-stabilized emulsions to develop bilayer emulsions, and the surface coating with gum arabic resulted in the smooth and bead-free nanofibers. To improve the mechanical properties of electrospun fibers and

to preserve fiber morphology when exposed to water, genipin was investigated as a safe cross-linking agent for gelatin-based electrospun fibers. These results contributed to a good understanding on the emulsion electrospinning of food materials for potential applications in bioactive encapsulation, enzyme immobilization and active food packaging.

Interactions between Peptides and Polyphenols: Their Potential Usages in Recovering Peptides Xiaonan Sui*, and Lianzhou Jiang, *Northeast Agricultural University, China*

Abstract not available.

Bioactivity of Anti-cancer Pentapeptide and its Application in Orange Juice Navam S. Hettiarachchy*, Ruiqi Li, and Ronny Horax,*University of Arkansas, USA*

A pure pentapeptide (Glu-Gln-Arg-Pro-Arg) derived from heat-stabilized rice bran was evaluated for its anti-proliferative activity against human cancer cells and applied in orange juice powder for its stability and sensory studies. Cell proliferation inhibition of the pentapeptide was evaluated using MTS assay. The molecular mechanism of action against breast cancer cells was investigated via apoptotic properties and the amount of molecular targets (COX-2, Bcl-2, p53, TNF-α, Fas, Bax and ErbB-2). The pentapeptide was incorporated into spray-dried orange juice powder and evaluated for sensory using a 9-point hedonic scale and storage stability. The peptide at 600 µg/mL caused 80, 75, 77, and 60% inhibition to the growth of colon, beast, liver, and lung cancer cells, respectively. Apoptotic features including morphological changes, DNA fragmentation, and caspases activation of breast cancer cells treated with pentapeptide were detected. The decrease in COX-2, Bcl-2 and ErbB-2 and increase in p53, TNF-α, Fas and Bax expression were observed at 72 h exposure of the cells to pentapeptide. These results suggest that apoptosis occurred through caspase-dependent pathway and cell death signal was escalated by down-regulating ErbB-2 and COX-2 expression. The orange juice powder retained a maximum of 90 and 70% pentapeptide (620 µg/mL) under refrigerated and ambient temperatures respectively over a 6-month storage. Consumer panelists liked the appearance, color and mouthfeel (score ≥6) of the reconstituted pentapeptide incorporated orange juice. This study showed that the pentapeptide has the potential as an alternative anti-cancer drugs and a functional food ingredient in food products.

Hydrolysis of Carioca Beans Protein and Some Functional Properties Francine Gomes Basso Los[1], Ivo Mottin Demiate[2], and Buddhi Lamsal*[1],[1]*Iowa State University, USA;* [2]*Ponta Grossa State University, Brazil*

This study aims to evaluate some functional properties of enzyme-hydrolysed Carioca bean (CB) proteins in various preparations. CB proteins, extracted at pH 8.5 solubilization and pH 4.5 isoelectric precipitation, were hydrolyzed with Bromelin 2000 GDU/g, at pH 7.0 and 55ºC. Two degree of proteins hydrolysis (DH), 5% (CBH-5%) and 8% (CBH-8%), were compared with no-enzyme controls (CBC-5% and CBC-8%) at the same pH and temperatures. The CB samples were compared with Carioca bean raw protein (CBRP), which were not subjected to enzyme, pH and temperature treatments. The same treatments were applied to Soybean protein (SBH-5%, SBC-5%, SBH-8%, SBC-8% and SBRP). The functional properties Emulsification Capacity (EC) and Foaming properties (foaming capacity – FC, specific rate constant – K and rate of liquid incorporation into foam - Vi) were performed at pH 7.0, 5.0 and 3.0. The highest EC among the evaluated pHs was shown by CBC-5% at pH 3.0 (793.65 g/g). At pH 7.0 all CB samples presented similar

EC that soybean samples, but at pH 5.0 and 3.0 CB samples showed higher EC than SB samples. All CB samples did not have significant difference at 5% level (Tukey test) for FC at pH 7.0, 5.0 and 3.0. However CB hydrolyzed samples had better results than control samples for foaming rate constant, that indicated foaming stability. These results shows that Carioca beans proteins presents a good potential to be used as ingredient in food industry.

PCP 4a: Processing and Non-food Applications of Proteins

Chairs: Keshun Liu, USDA, ARS, USA; Jianping Wu, University of Alberta, Canada; and Yonghui Li, Kansas State University, USA

Quality, Safety and Stability of Protein for Pet Diets: An Overview of Research Evaluating Protein Ingredients for Pet Food Applications Greg Aldrich*, *Kansas State University, USA*

Pet food is a growing segment of the animal food market with expected sales in the US exceeding $30 billion in 2018. The growth of this market is supported in large part by new products, much of which are based on novel or re-positioned protein ingredients. Concurrent with these novel ingredients is a demand for products with higher levels of protein. This is creating opportunities and challenges. Near term protein quality is the biggest hurdle. Longer term competition from other sectors of the human food, animal and fish food industries may become a limiting factor to sustainability of this industry. Raw material supply and processing effects the protein quality of traditional animal and vegetable-based protein ingredients used in the pet sector. Add to this the need for compliance with the food safety modernization act which can influence availability and quality. These ingredients have also been typically stabilized after processing by chemical additives to retain their nutritional value for extended periods. This is occurring simultaneous to pet food customers demanding natural products with limited processing. This review will provide an overview of research regarding protein quality assessments, identify shortcomings and strategies to overcome these challenges, and insights regarding stability concerns. Ideas about how to strike a balance between nutrition, safety, and shelf-life for this critical ingredient stream will be offered.

Peptide Antioxidants from Cereal Grain Co-products and Performances in Pet Food and Feed Yonghui Li*[1], and Ruijia Hu[2],*[1]Kansas State University, USA; [2]Kansas State University, Grain Science and Industry, USA*

Antioxidants are widely used in food, feed, and pet food industries to delay lipid oxidation and prevent quality deterioration. A rising trend in these manufacturers and consumers is the gradual replacement of synthetic antioxidants with naturally derived antioxidants. Cereal grain proteins, such as those from corn or sorghum, contain abundant antioxidative peptide sequences and structural domains; however, they are mostly buried within the protein's hydrophobic core and inaccessible to prooxidants, radical species, and transition metal ions to present their antioxidant functions. The objective of this research was to develop functional hydrolysates and peptides from corn, sorghum, DDGS, and CGM proteins through manipulated enzymatic hydrolysis to achieve desirable antioxidant performances in fats/oils, pet food, and feed products. Eleven commercially available proteases were initially screened for antioxidant production. Neutrase and Alcalase were found to be most promising with regards to antioxidant yield and DPPH radical scavenging activity. Selected antioxidants were further formulated into fish oil, poultry fat, pork fat, shortening, ground pork, dry dog food, and dry poultry feed at different levels, which were incubated under accelerated conditions in an oven, and POVs and TBARS were measured. The inclusion of peptide antioxidants significantly improved the oxidation stability of these systems and effectively inhibited lipid oxidation. Both POV and TBARS decreased with increased amount of antioxidants. This study demonstrated that cereal grain proteins are feasible sources for peptide antioxidant production and these antioxidants could be potentially used as alternatives to

synthetic antioxidants in improving the oxidative stability of various lipid-rich products.

Self-assembly of Peptides is Responsible for Nanoparticle Formation of Canola Protein Cruciferin Jianping Wu*,*University of Alberta, Canada*

Canola is a major oilseed in Canada. Canola meal after oil extraction is used mostly as animal feed with limited value-added applications. Cruciferin, a major canola protein, was used to prepare calcium-induced nanoparticles by a cold gelation method. The particles were spherical in shape with ~ 200 nm diameter and polydispersity index (PDI) of 0.2 to 0.3. The particles did not show toxicity to Caco-2 cells at concentrations of up to 2.5 mg/mL. The cell uptake of labelled nanoparticles was also observed using confocal microscopy after 6 h incubation with Caco-2 monolayer. Our studies indicated that cruciferin was broken down into peptides during particle preparation, which prompted us further investigate 1) what peptides exactly assembled the nanoparticles; 2) what were the main driving forces that contributed to this assembly; 3) whether the primarily β-sheet-containing cruciferin will generate peptides to form β-sheet-stabilized nanostructure. The purposes of the presentation were to elucidate the underlying mechanism of the particle development and to identify the sequence of peptides that are responsible for the nanoparticle formation. Nanoparticles developed from the study could find applications for improving the bioavailability of nutrients and nutraceuticals in humans and animals.

Fungal Fermentation of Oilseed Meals and Cereal Grains to Produce Protein-rich Ingredients for Aquafeed Bishnu Karki*[1], Jacob Zahler[2], Stephanie A. Wootton[2], Burgandy R. Roberts[2], Jason Croat[3], Michael Brown[4], and William Gibbons[2],*[1]Dept. of Biology and Microbiology, South Dakota State University, USA; [2]South Dakota State University, USA; [3]CTE Global Inc., USA; [4]Dept. of Natural Resource Management, South Dakota State University, USA*

The demand for protein-based diet is increasing with the increase in the world population. The rapidly growing aquaculture industry is an efficient platform for production of animal protein. However, the high cost of the aquafeed ingredient such as fish meal is inhibiting the economic feasibility of aquaculture operations. Thus, interest has grown in utilizing the plant-based protein sources as an alternative to fish meal. The plant-based proteins have their own limitations such as low levels of the protein, lack of essential amino acids, presence of high fibers, and anti-nutritional factors (such as glucosinolates in canola and carinata meals) which limit inclusion rates in aqua-diets. The fungal metabolic process has been found to be effective in degrading the fibers and antinutritional factors in feed while simultaneously concentrating the protein and amino acids levels. Our team at South Dakota State University (SDSU) is been exploring the potential of using several fungal strains in creating nutrient rich ingredients using submerged and solid states fermentation. The range of fungal strains (T. reesei, A. pullulans, R. oligosporus, N. crassa. P. variotti, and F. venenatum) were screened in different feedstocks including canola meal, carinata meal, guar meal, sorghum hominy and barley for their ability to reduce the amount of carbohydrates while increasing the protein and amino acid titers. The results showed an increase in protein level by up to 50% and reduction in glucosinolates by ~98% depending on feedstocks. Overall, the fungal incubation process was found to be effective in producing the alternative plant-based protein ingredients for aqua-diets.

Aqueous Extraction for Making Feed Proteins from Soybeans Keshun Liu*, *USDA, ARS, USA*

Defatted soymeal, a coproduct of modern soybean processing, is primarily used in feed for various

livestock and farm fish worldwide. Because the defatting process uses hexane, it suffers from safety concerns and negative environmental impact and fails to remove heat-stable antinutrients directly. Thus, research was conducted to develop soy protein ingredients with reduced heat-stable antinutrients using aqueous solvents instead of hexane. Raw soybeans were divided into two portions, one was ground into enzyme active flour and the other was oven-roasted and ground into roasted flour. Aqueous extraction was done using both concentrate and isolate approaches. The effect of the aqueous extraction on content and recovery rate of protein, oil, oligosaccharides, and phytate in resulting protein products were investigated. The concentrate approach was applicable to both enzyme-active and roasted flours. Protein concentrates produced had oligosaccharides and phytate contents significantly lower than the original flours (85-92% and 56-74% reduction, respectively). The isolate approach was applicable only to the enzyme-active flour. The resulting isolate had higher protein content, but lower protein recovery than the concentrate. Both products had similar oligosaccharide contents, but the isolate had phytate content even higher than the raw flour. With either approach, oil was not easily separated out. Instead it went either into the protein fraction using the concentrate approach or into the fiber and protein fractions using the isolate approach. Based on the composition and recovery of nutrients and antinutrients, the protein concentrate made by the aqueous process was suitable for feed application.

Alternative Oilseed Crops (camelina, cuphea, lesquerella, pennycress): Novel Protein Sources for Industrial Uses Mila P. Hojilla-Evangelista*, Roque L. Evangelista, Gordon W. Selling, and Mark Berhow, *USDA, ARS, NCAUR, USA*

Current interest in alternative protein sources is driven not only by the ongoing upward trend in global protein demand but also by the high potential for use in industrial markets other than food and feed. Pennycress (*Thlaspi arvense* L.), camelina (*Camelina sativa*), lesquerella (*Physaria fendleri*), and cuphea (*C. viscosissima* x *C. lanceolata*) are non-traditional oilseed crops that contain substantial protein in the seeds (33, 44, 23, and 20 % db, respectively). We investigated the proteins from these four crops for composition, amino acid profile, extractability, and functional properties [solubility, foaming, emulsification, water-holding capacity (WHC) or heat coagulability]. SDS-PAGE revealed that the seed proteins of the crops were not large, as all polypeptide bands resolved below the 100 kDa marker. Cuphea protein was made up primarily of glutelin and showed highest solubility (> 90 %) at pH 10. Lesquerella protein contained mainly glutelin and globulin, were most soluble (> 60 % soluble proteins) at pH 2 and 10, and also had high WHC (8 g water/g protein). Camelina protein had acid-glutelin and albumin as major fractions. Pennycress seed protein (predominantly albumin and globulin) had poor solubility but excellent foaming and emulsifying capacities. The isolate form of pennycress seed protein (92 % db crude protein) is much more soluble (> 80 % from pH 2-10) and also heat-stable. Protein-mucilage material is also obtained from lesquerella, camelina, and pennycress. These new proteins have properties that are desirable for pressurized foams, emulsions, viscosity agents or adhesives.

PCP 4b: General Protein and Co-Products

Chairs: Nandika Bandara, Dept. of Plant, Food & Environmental Sciences, Dalhousie University, Canada; and Apollinaire Tsopmo, Carleton University, Canada

Identification of a New Potential Allergen from Mullet and Salmon Qinchun Rao*, and Behnam Keshavarz,*Florida State University, USA*

Objective: To identify potential allergens from mullet and salmon using human immunoglobulin-E (IgE) from patients with a history of fish allergy. Methods: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western bot (WB) were applied to determine the protein extracts (PE) profile and to identify IgE-binding proteins using seven fish allergic human sera, respectively. Pepsin digestion stability of tropomyosin was performed using pooled human IgE and an anti-tropomyosin polyclonal antibody (pAb) in WB to evaluate the antigenicity of digested tropomyosin. IgE epitopes were also mapped for salmon tropomyosin using the SPOT synthesis technique. Results: Parvalbumin found to be the major allergen in all tested sera. Tropomyosin-bound IgE was identified in five of seven tested sera (about 71%). The sera containing specific IgE reacted to tropomyosin, suggesting its cross-reactivity with tropomyosins from these two species. MS analysis further confirmed the presence of tropomyosin in mullet and salmon. Pepsin digestion stability also showed that IgE epitopes of tropomyosin from mullet and salmon were stable up to 8 min and 4 min, respectively. Moreover, IgG epitopes of tropomyosin from mullet and salmon were stable up to 15 min, and 8 min, respectively. Two distinct regions on salmon tropomyosin were found to contain IgE epitopes. Conclusions: To the best of our knowledge, this is the first report of tropomyosin as an IgE-binding protein from mullet and salmon.

Antioxidant Activity in Amaranthus hypochondriacus Protein Fractions Fermented with Lactic Acid Bacteria at Different Growth Stage Apollinaire Tsopmo[1], Fabiola Sánchez*[2], Madeleine Morales[3], and Víctor J. Robles[3],*[1]Carleton University, Canada; [2]Instituto Tecnológico de Veracruz, Méxio; [3]Instituto Tecnológico de Veracruz, Mexico*

Objective. Amaranthus protein fractions were treated with lactic acid bacteria at various stage of growth followed by an evaluation of their antioxidant properties. Methods. Defatted amaranth flour was used to extract reserve proteins (albumins, globulins (G7S, G11S) and glutelins) based on their solubility. The protein concentration was determined and characterized by SDS-PAGE electrophoresis. Each protein fraction was treated with Lb. plantarum (LP) and Lb. helveticus (LH) harvested at different growth phases. Free amino acid contents, radical scavenging (ROO•, HO•) capacities and zinc chelation were determined for the hydrolysates. Results. Glutelins and albumins had the highest protein contents 0.96 ± 23.48 and 0.90 ± 6.20µg/mL. Bacteria used to hydrolyze each protein fraction were harvested at 4, 8, 14 and 24h. Hydrolysates with the highest protein concentrations were glutelins treated with LH collected at 14h (147 µg/mL). The highest degree of proteolysis was observed at 24h in the albumin fraction treated with cells of both strains. The highest ROO• activities for albumins at 8h with LP (1037 ± 79.55 µM TE/mL). The highest percentages of •OH and zinc-chelation were obtained with the hydrolysates of fraction G11S at 24h with LP and 48h with LH. Conclusion. The growth stage Lb. plantarum and Lb. helveticus has an influence on the activity of A. hypochondriacus

protein fractions and this can be useful in the production of a hydrolysates with biological function.

Alternative Method of Obtaining Amino Acids from Canola Meal for Further Conversions as Functional Molecules Sumudu N. Warnakulasuriya*[1], Takuji Tanaka[1], and Janitha P.D Wanasundara[2],[1]*University of Saskatchewan, Canada;* [2]*Agriculture and Agri-Food Canada, Canada*

The non-reversible structural changes occur in proteins during industrial canola oil extraction process consequently affect the nutritional and functional value of meal proteins. Present study investigated recovery of amino acids from canola meal (CM) via acid hydrolysis for new, functional molecule generation. Expeller pressed (EP) CM (34% protein, 13% oil) was pre-treated with ethanol (99%, v/v) to remove oil and small molecules, and subsequently alkali (pH 12) treated to obtain concentrated protein. Acid hydrolysis of the meal protein was carried out with H_2SO_4 (3M, 2M, 1M) and compared with standard hydrolysis conditions; 6M HCl, 24 h at 110°C. Extent of peptide bond cleavage expressed as degree of hydrolysis (DH) was determined by released free amino groups using *o*-phthalaldehyde assay. Ethanol treatment concentrated protein up to 39% (w/w) and alkali treatment further improved it up to 64% (w/w). Without ethanol treatment, alkali treatment alone achieved 49% protein enrichment. Untreated meal and ethanol treated meal needed 3M H_2SO_4 to achieve the highest DH (100%). The meal underwent only alkali treatment gave 89% and 77% of DH at 3M and 1M concentrations, respectively. However, the meal treated under both ethanol and alkali conditions showed no significant differences in DH with lowering the acid concentration from 3M to 1M. Results indicated that pre-treatment of CM with ethanol and then alkali supports efficient release of amino acids under much lower acid concentration than usually used for total hydrolysis. Conversion of CM derived amino acids into N-acylated molecules and their applications are discussed.

Speciation of Arsenic and Chromium in the Presence of Hydrolyzed Oat Proteins Apollinaire Tsopmo*,*Carleton University, Canada*

Transition metals play an important role in a wide variety of biological processes in living systems but this is dependent on the quantity and the type of species present. Specific forms of arsenic (As) and chromium (Cr) are associated with oxidative stress, cellular damage and inflammation. The aim of this work was then to test in a food system whether, in the presence of hydrolyzed oat proteins, arsenic or chromium will exist predominantly in a specific oxidative state and evaluate the potential implication of in promoting or decreasing oxidative stress. Eight hydrolyzed proteins were produced by combining two extraction methods and four proteases. The addition of hydrolysates to ground meat decreased lipid hydroperoxides by up to 50% when stored at 4 ºC but had no effect at – 20 ºC. The ratio of As(V) to As(III) in meat was about 2:1 but the presence of hydrolysate the amount of As(III) detected was 3-fold higher depending on storage conditions. This was due to better extraction of As(III) in the presence of hydrolysates rather than to the reduction of As(III) since the concentration of As(III) was similar between control and treatment groups. Data on chromium showed Cr(VI) decreased from 1.43 ± 0.4 to 0.63 ± 0.05 µg/g while concentrations of Cr(III) increased from 0.28 ± 0.02 to 0.86 ± 0.07µg/g. In summary, the addition of hydrolyzed oat proteins had little effect on As speciation during storage but they appeared to reduce Cr(VI) to Cr(III)

Protein Based Delivery Systems for Improved Bioavailability of Bioactives: Past, Present and Future Nandika Bandara* and Thilini Dissanayake, *Department of Plant, Food and Environmental Sciences, Dalhousie University, Nova Scotia, Canada*

Bioactive compounds gained enormous research interest in the past due to their unique abilities in managing and preventing chronic disease conditions. However, nutraceutical applications of these bioactives are hindered due to extraction and processing induced poor bioavailability, poor systemic delivery and efficacy. Several technologies including nanoencapsulation has been extensively explored to improve these properties. Among these technologies, protein-based delivery systems have gained a considerable attention due to their versatile binding ability, biodegradability, minimum toxicity and food grade compatibility. Whey protein, gelatin, casein, prolamine, wheat germ agglutinin, albumins, zein, pea protein, soy protein and several other proteins has been used to develop delivery systems for encapsulating bioactives. Recently, protein based dual polymer systems such as protein-polysaccharides and protein-lipid systems have gained the traction in encapsulation research to harness functional properties of both polymers for developing advanced delivery systems. In this review, we will critically evaluate the most significant achievements in the protein-based delivery systems to date, key challenges exist, and how the future research should be directed in order to develop advanced delivery systems for bioactive encapsulation.

PCP-P: Protein and Co-Products Poster Session

Chairs: Bishnu Karki, Dept. of Biology and Microbiology, South Dakota State University, USA; and Mila Hojilla-Evangelista, USDA, ARS, NCAUR, USA

1. ***In vitro* Antioxidant and Lipase Inhibitory Activities of Oat Bran Derived Peptides.** Apollinaire Tsopmo, and Ramak Esfandi****(Protein and Co-Products Division Student Travel Grant Winner)***, *Carleton University, Canada*

The accumulation of fat in the human body is generally accompanied by an increase in the body weight and also by an imbalance in the oxidation-reduction system. The incorporation of bioactive compounds in the human diet may help to overcome some of the complications related to excess weight through an antioxidant mechanism or regulation of enzymes involved in lipid absorption or metabolism. In recent years, special attention has been paid to food-derived bioactive peptides specifically to the ones with multifunctional activities. This study then aimed to determine the ability of recently identified oat bran peptides to quench radical species, retard the oxidation of lipids, and to inhibit pancreatic lipase. The peptides tested were P1: YFDEQNEQFR, P2: GQLLIVPQ, P3: SPFWNINAH, P4: NINAHSVVY, and P5: RALPIDVL. Data showed that P3 (1.6 ± 0.1 µmol Trolox equivalents (TE)) and P4 (1.7 ± 0.3 µmol TE) highly quenched ROO• radicals. HPLC data also showed that highest activities of P3 and P4 were due to their greater reactivity with the peroxyl radical as 96% by P3 was consumed compared to for example 11% for P2 the least active. In the lipase inhibitory activity, all peptides at 50 µM affected the activity. Peptide P1 had the highest inhibition (46.7 ± 1.1%) followed by P4 (33.5 ± 2.3%). These finding demonstrated a potential use of the stated peptides in the decrease of oxidative stress and the control of lipid absorption.

2. **Effect of Sonication on Extraction of Proteins from Oats and Antidiabetic Properties of Hydrolysates.** Mallory E. Walters****(Protein and Co-Products Division Student Travel Grant Winner)*** and Apollinaire Tsopmo, *Carleton University, Canada*

The aim of this study was to determine the effects of extracting proteins under normal (CTL), ultrasonic bath (UB) and high power (HP) ultrasonic conditions on the biological activity of hydrolysates. Mass spectrometry data showed that CTL, UB and HP samples contained some identical globulin and avenin proteins. Meanwhile, vromindoline, a starch-bound protein, was only detected in samples subjected to ultrasounds (UB, HP), while tryptophanin, a lipid binding protein, was present only when HP ultrasounds were applied. The anti-diabetic activity of hydrolysates (Flavourzyme, Papain, Alcalase) from each extraction method was evaluated through their ability to modulate the activity of dipeptidyl-peptidase-4 (DPP-4) and alpha-amylase, as well as the secretion of GLP-1, a hormone that enhances insulin response in NCI-H716 cells. Ultrasonication did not affect DPP-4 inhibition; however, concerning proteases, Papain hydrolysates had substantially greater (49.7 - 53.6%) DPP-4 inhibitory activity for all conditions. HP sonication slightly decreased the alpha-amylase inhibitory activity by 4.8 – 7.2% compared to UB and control. Cellular viability assay showed that hydrolysates at concentrations 0.2 – 0.8 mg/mL maintained the integrity of the colon NCI-H716 cells. Overall, the effect of ultrasonic treatments on the composition of proteins obtained from oats was the release of two proteins not found in the control. The enzymatic hydrolysis of protein isolates with proteases produced hydrolysates with notable anti-diabetic activity (DPP-4 and alpha-amylase

inhibition) but this was not influenced by the difference in polypeptide compositions.

3. **Yellow Bean: Processing, Characterization and Product Development.** Jacquiline N. Maina[1], Anh T.L Nguyen[2], Peace C. Asuzu[3], Samuel A. Besong[4], and Alberta N.A. Aryee*[2], *[1]EARTH University, Costa Rica; [2]Delaware State University, USA; [3]College of Agriculture & Related Sciences, Delaware State University, USA; [4]Dept. of Human Ecology, College of Agricultural Sciences, Delaware State University, USA*

Beans are important sources of proteins, carbohydrates and minerals, but remain underutilized in product development. The present study explored the use of common processing treatments and determined their effects on the physicochemical characteristics, nutritional quality and functional properties of yellow beans to increase applications in product development. Yellow kidney bean, also known as African yellow bean (AYB) or heirloom beans, was subjected to milling, sieving, soaking, cooking, blanching, roasting, fermentation (natural and yeast) and, lastly, alkaline extraction and isoelectric precipitation. Notable differences were found in the proximate composition of the various processed flours. Fermented flours had higher amount of total phenolic compounds (0.91 - 0.93 mg GAE/g) than the other flours (0.52 - 0.63 mg GAE/g). Phosphorus (P) content was used as an indicator of phytic acid content and values were 7.50% P in the fermented flours and 8.60% P in the cooked flour. Blanching and fermentation produced flours with higher fat absorption capacity (2.00 – 2.08 g/g) compared with the other treatments (1.68 – 1.95 g/g). Cooking and soaking increased the water holding capacity and water absorption index by more than 45%, compared with the fermented flours. Roasted flours had the highest amount of riboflavin (20.95 mg/g) and thiamin (4.07 mg/g) compared with the other flours. Boiling, roasting and fermentation produced flours with lower swelling power, as well as reduced foaming and emulsification properties compared with the other treatments. Milling, roasting, blanching and fermentation affected the color, aroma, fluidity, consistency, texture, astringency, aftertaste, and overall acceptance of formulated hummus. This study shows that simple and common processing treatments can be used to develop yellow bean flour with diverse properties, improve industrial opportunities and find application in food, beverages and dips.

4. **Value Added Use of Commercial Canola Meal Protein via Converting into Amino Acids.** Sumudu N. Warnakulasuriya*[1] ***(Protein and Co-Products Division Student Travel Grant Winnerz)***, Janitha P.D Wanasundara[2], and Takuji Tanaka[1], *[1]University of Saskatchewan, Canada; [2]Agriculture and Agri-Food Canada, Canada*

Value addition to commercial canola meal (CCM) needs alternative ways beyond using functional and nutritional properties of its proteins. Converting amino acids into useful functional molecules has been reported as a feasible process. The objective of this study was to examine the pre-treatments and hydrolytic conditions that can provide the maximum amino acid yield from CCM as a protein source. Residual oil and small molecules (mainly phenolics) of CCM were removed by treating with ethanol [99% v/v, at 50oC, meal-to-solvent ratio of 1:4 (w:w) for 30 min] to facilitate protein extraction and hydrolysis. The treated meal was alkali-treated (pH 12, 1 h, ambient temperature with mixing) to extract proteins. Acid hydrolysis of freeze-dried proteins was carried out with H2SO4 (3 M, 2 M, 1 M). Reference acid hydrolysis conditions were: 6 M HCl, 24 h at 110oC. The ethanol pretreated meal yielded products with protein content to 50% (w/w). The acid hydrolysis of the untreated

meal and ethanol-treated meal needed 3 M H2SO4 to achieve the highest degree of hydrolysis (DH). The alkali-extraction further enhanced the recovery of amino acids. The protein concentrate extracted from untreated meal gave 96% and 80% of DH at 3 M and 1 M acid concentrations, respectively; whereas, the protein concentrate obtained through a combination of ethanol-treatment and alkali extraction gave 100% DH with 1 M H2SO4. Pretreating the meal, to remove nuisance molecules prior to alkali extraction, facilitated achieving a higher yield of amino acids at lower H2SO4 concentration, demonstrating a cost-effective process.

5. **Utilizing pretreatments and fungal fermentation to enhance the nutritional profile of distillers' dried grains with solubles (DDGS) for Aqua-diets.** Burgandy R. Roberts*[1], Bishnu Karki[2], Michael Brown[3], and William Gibbons[1], *[1]South Dakota State University, USA; [2]Dept. of Biology and Microbiology, South Dakota State University, USA; [3]Dept. of Natural Resource Management, South Dakota State University, USA*

With the increasing demand for renewable energy, corn ethanol had been developed to fill the need. As a result of corn ethanol production, dried distillers' grains with solubles (DDGS) has become an inexpensive feed for several animals. However, due to the presence of high amount of fibers and low protein content of DDGS, it's use in the animal diet is limited to less than 30% on dry basis. In this study, fungal metabolic process was used to determine if nutritional composition of DDGS could be improved by increasing the protein levels and reducing the fibers and phytics content in the DDGS..Four different fungal strains (T. reesei (NRRL-3653), N. crassa (NRRL-2332), R. oligosporous (NRRL-2710), and A. pullulans (NRRL-Y- 2311-1)) at three solid loading rates (SLR) (5%, 10%, and 20%) were tested. The protein titers ranged from 35 to 37% irrespective of the microbes used. As expected protein titers were slightly decreasing with the increase in SLR. The crude fiber content was increased as compared to that of the un-inoculated control due to the concentration effect. Whereas phytic acid level was decreased in the fermented DDGS as compared to that of the control. To determine if the protein titers could be enhanced by adding the enzymes prior to fungal fermentation, range of commercial enzymes (galactosidase, xylanase, pectinase and cellulase) from BioCAT are currently being evaluated.

6. **Role of Intracellular Protein Fraction of Lactobacillus casei CRL-431 on its Bioactive Properties.** José E. Aguilar-Toalá*[1], Hugo S. Garcia[2], Andrea M. Liceaga[3], Belinda Vallejo-Cordoba[1], Aarón F. González-Córdova[1], and Adrian Hernández-Mendoza[1], *[1]Centro de Investigación en Alimentación y Desarrollo, A.C., Mexico; [2]Technological Institute of Veracruz, Mexico; [3]Purdue University, USA*

Different studies have shown that metabolites produced by lactic acid bacteria (LAB) have antioxidant activity. Among a variety of metabolites, extracellular proteins, and small peptides secreted by LAB have been previously characterized. However, information regarding the role of the soluble intracellular protein fraction, and its possible interactions with other intracellular components, on antioxidant properties of LAB is still scarce. In this study we found a significant (P CRL-431 or its intracellular content (IC-CRL431), respectively. Furthermore, a synergistic interaction was observed between glutathione (GSH) and the lipid or proteinaceous fraction. Besides, GSH-lipid-proteinaceous fractions showed an additive interaction, while an antagonist interaction between lipid and proteinaceous fraction was observed. On the other hand, the IC-CRL431 exhibited angiotensin converting-enzyme

inhibition activity (93.6%). These results suggest that soluble intracellular protein fraction may play an important role on the antioxidant properties of Lactobacillus casei CRL-431 and have a potential antihypertensive effect. Therefore, open up another strategy that should be explored towards the discovery of new metabolites with multifunctional biological activities from the intracellular content of LAB.

7. **Keratin Protein Derived Nanomembrane for Water Purification.** Muhammad Zubair*, Roopesh Mohandas, and Aman Ullah, *University of Alberta, Canada*

One of the most serious global challenges is inadequate access to fresh water, that is predicted to grow worse in the future as demand continues to rise due to ever increasing world population, rapid industrialization and greater energy needs. Clean water is essential to protect human and any other life on the planet earth. However, one tenth of the global population do not have access to safe drinking water. Conventional approaches such as reverse osmosis, decontamination and disinfection can address many water problems. However, these methods are often chemically, energetically and operationally intensive and, thus require considerable infusion of capital. Herein, we are proposing the development of an integrated low cost, robust and efficient water treatment technology based on keratin proteins/ graphene oxide with a potential to remove metals, organics and pathogens in a single treatment without further stressing environment. In this study, graphene oxide was prepared and characterized using XRD, TGA, FTIR techniques. For the synthesis of keratin/GO derived nanomembrane, keratin proteins have been extracted from chicken feather by the removal of lipids followed by using reducing agents. Then, extracted keratin and graphene oxide powders was compressed molded using compression molding technique. Furthermore, the membrane properties will be evaluated with XRD, AFM, Raman spectroscopy, XPS, solid state NMR and TEM. This study can open up new horizons to exploit unique properties of both chicken feathers proteins and graphene oxide for water purification.

8. **Effect of Demucilaging Methods on Functional Properties of Flaxseed Protein Isolates from Flaxseed Cake.** Yang Lan* ***(Protein and Co-Products Division Student Travel Grant Winner)*** and Jiajia Rao, *North Dakota State University, USA*

Application of flaxseed in the food industry is primarily limited to its oil and the whole seeds. The interference of flaxseed mucilage in protein isolation and the lack of effective extraction methods have prevented the broader utilization of flaxseed protein isolate (FPI). This study aimed to investigate the effect of demucilaging treatments, such as warm water (W-FPI) and enzymatic method (E-FPI) on physicochemical properties of FPI from flaxseed cake. The structural (amino acid composition and molecular weight (Mw)) and functional properties (solubility, surface charges, foaming, and emulsifying properties) of W-FPI and E-FPI were determined. The results indicated that functional properties of FPIs were significantly influenced by demucilaging methods. In general, both FPIs showed a limited range of Mw bands in range of 10-50 kDa with the highest proportion of Mw at 25-30 and 35-40 kDa. The amino acid profile of FPIs showed relatively high amount of arginine, aspartic acid and glutamic acid while low amount of cysteine and methionine. The solubility of W-FPI was slightly higher than that of E-FPI at neutral pH. Two protein isolates showed similar surface charge magnitude throughout pH 10-2, ranging from -36 to 16.7 mV, and charge neutralization at pH 4.2. For both isolates, foaming capacity was significantly higher at acidic pH than neutral pH while foam stability was similar at

both acidic and neutral pH. This study showed that different functional properties of FPI could be achieved using the different preparation methods, leading to potential use in food systems with designed functional properties.

9. **Complex Coacervation of Pea Protein Isolate with Sugar Beet Pectin for Controlling Food Texture.** Yang Lan* and Jiajia Rao, *North Dakota State University, USA*

One of challenges for incorporation of plant-based proteins into foods is an unacceptable food texture and structure. Complex coacervation between pea and polysaccharide often results in the formation of a variety of complex structures that give rise to diverse textural attributes. This work aimed to study the formation and mechanism of complex coacervates of pea protein isolate (PPI) and sugar beet pectin (SBP). The effects of pH (2-7) and PPI-SBP mixing ratios (1:1 to 20:1) on formation and phase behavior were investigated by phase diagram, zeta potential, Fourier transform infrared spectroscopy (FTIR), isothermal titration calorimetry (ITC), scanning electron microscopy (SEM) and dynamic rheological analysis. Zeta potential, FTIR and ITC results revealed that there were a hydrogen bonding and electrostatic interaction between PPI and SBP. The complex coacervation was spontaneous exothermic reaction which was driven by negative enthalpy changes (-68 kJ/mol). As the PPI-SBP mixing ratios increased, the pH for complex coacervate formation shifted to higher values. Rheological results indicated that all coacervates showed higher storage modulus (G') than loss modulus (G"), and G' was relatively higher at mixing ratio of 20:1, 10:1 and 5:1. The coacervates formed at pH 3.5 and 2.5 both displayed a sponge-like porous network microstructure at relatively higher PPI-SBP mixing ratio, which suggested that interconnected gel-like structure can be obtained at higher mixing ratios. Our results provide a deep understanding of the mechanisms of complex coacervation between globular proteins and anionic polysaccharides.

10. **Bioactive Properties of Hairless Canaryseed Protein.** Emily Mason*[1] ***(Protein and Co-Products Division Student Travel Grant Winner)***, Lamia L'Hocine[2], Allaoua Achouri[2], Melanie Pitre[3], and Salwa Karboune[4], *[1]McGill University, Canada; [2]Agriculture and Agri-Food Canada, Canada; [3]Agriculture and Agri Food Canada; [4]Dept. of Food Science and Agricultural Chemistry, Faculty of Agricultural and Environmental Sciences, McGill University, Canada*

Hairless canaryseeds are a novel cereal grain recently approved for human consumption in Canada and the United States. To date, the limited research conducted on canaryseeds has been done on the previous non-edible hairy canaryseeds. The objective of this study was to determine the in vitro bioactive properties of hairless canaryseed protein and compare them to those of two conventional cereal grains, wheat and oat, in order to ascertain their health promoting effects. Four different cultivars of Canadian produced glabrous canaryseeds (two yellow and two brown) and one commercial cultivar of wheat and oat were digested following the harmonized INFOGEST in vitro protocol. The hydrolysates from the in vitro digestion were ultra-filtered, and the permeates were subsequently screened for their antioxidant activity (ORAC, DPPH, and ABTS assays), iron chelating activity (Fe2+ chelation assay), antidiabetic activity (DPP-IV inhibition assay), and antihypertensive activity (ACE inhibition assay). Overall, hairless canaryseeds demonstrate excellent bioactivity, particularly antihypertensive activity. Canaryseed antioxidant, antidiabetic, and iron chelation activities were comparable or greater than oat and wheat. Yellow canaryseed cultivars possess

higher antihypertensive activity than both oat and wheat. Not only are hairless canaryseeds high in protein (23%), but they demonstrate high potential for health improvement and prevention of nutrition related chronic diseases.

11. **L-cysteine Effects on Chlorogenic Acid Quinone and Amino Acid Induced Greening and Maillard Browning.** Yundi Liang* and Lilian M. Were, *Chapman University, USA*

The use of sunflower meal can be limited by formation of green trihydroxy benzacridine (TBA) derivatives between chlorogenic acid (CGA) quinones and amino acids. To investigate an amino acid-based de-greening approach, L-cysteine was studied in L-lysine and CGA mixtures because CGA-cysteine conjugates are colorless. Buffered solutions of 11.2 mM L-lysine: 5.6 mM CGA: 0-180 ppm L-cysteine (ratio 1:1:0.2) were incubated at pH 7.75, 8.0 and 9.0 for 48 h at ambient temperature. Color, reducing capacity, and LC-MS conjugates were tested after 1 to 8, 24 and 48 h of incubation. In pH 8.0 buffered solutions, L-cysteine concentration was positively correlated with browning, but had opposite effect on greening. After 24 and 48 h, greening decreased with increasing L-cysteine: $r = -0.41308$, $p = 0.0322$ and $r = -0.89311$, $p < 0.0001$ respectively. Both greening and browning significantly decreased with a 0.26 pH reduction to 7.75. At pH 9, both browning and greening were strongly negatively correlated with L-cysteine over time. In CGA quinone-amino acid solutions, the lower pH affected browning to a greater extent than greening. Increased reducing capacity was observed with increasing L-cysteine as pH was lowered from 9.00 to 7.75. Enhanced browning was attributed to oxidation of dimerized CGA to brown-yellow CGA quinones at alkaline pH. Reduced greening with L-cysteine was attributed to cysteine reacting with CGA faster to form colorless compounds before CGA reacted with lysine to form TBA derivatives, which was confirmed with LC-MS conjugates. Cysteine is thus a suitable anti-greening agent.

12. **Enzyme-assisted Aqueous Extraction of Oil and Protein from Almonds and Cream De-emulsification.** Fernanda Furlan Goncalves Dias*, Thaiza Serrano Pinheiro de Souza, and Juliana M. Leite Nobrega de Moura Bell, *UC Davis, USA*

The enzyme-assisted aqueous extraction process (EAEP) is an environmentally friendly strategy that enables simultaneous extraction of oil and protein from many food matrices. The aim of this study was to evaluate the effectiveness of proteases to assist the extraction of oil and protein from almond flour and its impact on in vitro protein digestibility. In addition, enzymatic [using neutral protease (NP2M, Bio Cat)] and chemical (isoelectric point precipitation, pH 5.0) treatments were evaluated to de-emulsify the oil-rich cream from AEP and EAEP. EAEP and AEP had oil extraction yields of 67 and 62%, respectively, while protein yields were 76 and 65%, respectively. Except for the use of protease in EAEP (0.5% w/w), extraction parameters were similar for both processes (pH 9.0, 50 ºC, 1:10 solids-to-liquid ratio, 1 h). A fractional factorial design was used to evaluate the effects of pH (6.0-9.0), temperature (50-65 °C), time (30-90 min), and amount of enzyme (0.5-2.5% w/w) on the enzymatic de-emulsification of AEP and EAEP creams. Subsequently, a central composite rotatable design was performed to identify optimum de-emulsification conditions. Chemical de-emulsification of AEP and EAEP creams resulted in oil recoveries of 65 and 74%, respectively; while enzymatic de-emulsification led to oil recovery yields of 64 and 90%, respectively. The EAEP skim had higher in vitro digestibility compared with the AEP skim (88 vs. 80%). Overall, the use of enzyme during extraction improved oil and protein extractability, facilitated the de-emulsification

of the cream fraction, and produced a protein fraction (skim) with higher in vitro digestibility.

13. **Effect of Growing Conditions on the Protein Digestibility and the Production of Peptides with Antioxidant Activity of Locally Cultivated Great Northern Beans (*Phaseolus vulgaris*).** Madhurima Bandyopadhyay* and Kaustav Majumder, *University of Nebraska, Lincoln, USA*

Dry edible beans are an excellent source of dietary proteins and biologically-active peptides with health benefits. However, different growing conditions may modulate the functionality and biological properties of the bean-derived proteins and peptides. Therefore, the primary objective of the present study is to evaluate the effect of growing conditions on protein digestibility and the antioxidant activity of the whole bean hydrolysate. Great Northern beans grown in both drought and irrigated conditions were first soaked overnight at 4oC. The soaked beans were cooked at 95oC for up to 90 min and hydrolyzed by Alcalase for 3 h. Antioxidant capacity of the whole bean hydrolysate was measured by the ABTS (2,2'-azino-bis-3ethylbenzothiazoline-6 sulphonic acid) radical scavenging assay. The beans grown in drought conditions have significantly less amount of protein (18.9 %) compared to the beans grown in irrigated conditions (22.8 % protein content). However, the drought-beans tend to show a slightly higher degree of hydrolysis (DH:19.2% ± 1.9), compared with irrigated-beans (DH: 16.9% ± 1.2). Furthermore, the ABTS assays showed an IC50 value of 0.8 mg/mL for the drought-bean-hydrolysate and 0.7mg/mL for the irrigated-bean-hydrolysate. Thus, the results obtained from the study so far indicate that the Great Northern beans grown in drought conditions, despite having a lower amount of protein, had exhibited no significant difference in Alcalase digestibility and antioxidant activity compared to the beans grown in irrigated conditions.

14. **Influence of Processing Conditions on the Structure, Functionality and Flavor Profile of Pea Protein Isolate.** Zili Gao*[1], Peiyi Shen[2], and Jiajia Rao[1], *[1]North Dakota State University, USA; [2]Dept. of Plant Sciences/North Dakota State University, USA*

The common method for preparing the pea protein isolate (PPI) involves alkaline extraction followed by an isoelectric precipitation. Nevertheless, these studies have focused on isolation of PPI under one condition and evaluated their functionality at neutral pH. There is a lack of systematic studies indicating how isolation parameters such as alkaline pH impact their yield, functionality and flavor profile. The aim of this study was to optimize alkaline extraction for production of PPI with desired flavor profile and functionality. The impact of extraction pH on the molecular structures, lipoxygenase activity of PPI was investigated using SDS-PAGE and circular dichroism (CD) spectrum. The SDS-PAGE profiles and CD data of the PPI obtained from different pH showed large similarities with the major storage proteins of legumin, vicilin and convicilin. The results demonstrated that the protein yield increased by 15% as the pH increased from 8.5 to 10. However, extended lipoxygenase activity and lower solubility were observed as the pH increased from 9 to 10. Moreover, GC-MS profile of PPI indicated that extraction at pH 9 resulted in the lowest amount of beany flavor compounds, including 1-butanol, 3-methyl, 1-pentanol, hexanal, 1-octen-3-ol, 1-octen-3-one, 2-octenal, (E)-, acetopheone, 2-nonenal, (E)- and pyrazine, 2methoxy-3-(1-methylethyl). Therefore, a solid/solvent ratio of 1:15 (g: mL) and pH 9 were found to be optimal extraction conditions for yield, functionality and flavor profile. The information obtained from this study may be useful for producing PPI with enhanced flavor

and functionality best suited for a specific end use.

15. **Bioprocessing Affects Seed Microstructure, Phenolic Compound and Protein Profiles of Yellow Pea and Green Lentil.** Chibuike C. Udenigwe[1], Apollinaire Tsopmo[2], Teresa Oliviero[3], and Elisa Di Stefano*[1]***(Protein and Co-Products Division Student Travel Grant Winner)***, *[1]University of Ottawa, Canada; [2]Carleton University, Canada; [3]Wageningen University, Netherlands*

Yellow pea and green lentils are important sources of proteins and phenolic compounds, although these beneficial components may not be bioaccessible during digestion. Metabolic activities during germination and solid-state fermentation (SSF) can affect de novo synthesis and bioconversion of phenolic compounds and proteins. In our study, we investigated the effect of germination and SSF with Lactobacillus plantarum on the content and profile of phenolic compounds and proteins in yellow peas and green lentils. Phenolic extracts were profiled by HPLC-DAD, and proteins profiles were analyzed by SDS-PAGE. The pea proteins, convicilin, vicilin and provicilin, disappeared after three days of germination, while SSF led to degradation of lipoxygenase, convicilin, legumin and albumin. Regarding the phenolic profile, both germination and SSF led to a decrease in major flavonoids, kaempferol tetraglycoside and triglycoside, and an increase in kaempeferol glycoside in green lentils after SSF. In yellow peas, germination led to an increase in protocatechuic acid, vanillic acid, chlorogenic acid and flavonoid derivatives. Cell wall integrity analysis using brightfield and fluorescence microscopy revealed a significant degradation of the cell wall of both legume seeds after SSF. Germination caused an increase in cell wall thickness and depletion in starch and protein granules. Therefore, germination and SSF altered the content and profile of proteins and phenolic compounds in green lentils and yellow peas. The results of our study provide evidence for the use of these bioprocessing techniques for tailoring specific compound profiles for the development of nutrient-rich and health promoting foods.

16. **Development of Sandwich Enzyme-Linked Immunosorbent Assay for Porcine Hemoglobin Quantification.** Xingyi Jiang*, Qinchun Rao, Meng Wu, and Weiya Dong, *Florida State University, USA*

Objectives: (1) Characterization of monoclonal antibodies (mAbs) and rabbit sera (SR); (2) development of indirect sandwich enzyme-linked immunosorbent assay (sELISA) for porcine hemoglobin (PHb) quantification. Methods: Dot blot was performed to study the selectivity of mAbs and SR. Fluorescent Western blot (WB) was performed to study PHb immunoreactive subunit. PHb extractability using different buffers were compared using blotting. During assay development, each step was optimized using ELISA. A standard curve was established to quantitatively determine PHb amount in laboratory-adulterated samples. Results: First, according to dot blot, mAb2 showing the best selectivity that it could only react with hemoglobin from the porcine blood. From fluorescent WB results, mAb2, SR1, and SR4 all reacted with PHb-β subunit. Further ELISA checkerboard study showed that mAb2 and RS1 provided the better signal. Second, as a cytoplasmic protein, PHb showed a better extractability in buffers with an ionic strength less than 50 mM. An alkaline pH provided improved stability and the best extraction results. During assay development, the results showed that medium binding microplate could reduce non-specific binding. In addition, a one-step incubation of detection antibody and secondary antibody reduced assay time. A standard curve was established. The limit of detection was 31 ppm, and EC50 was 81 ppm.

The detection range was 62 – 2000 ppm. Conclusion: A sELISA was established to quantitatively determine PHb in animal meat. It can be used for (1) monitoring bleeding efficiency and pork quality; (2) identification of kosher and halal foods; and (3) species authentication.

17. **Comparing the Glyceollin Production Efficiency of Different Varieties of Soybeans during Fungal Infection.** Bishnu Karki*[1], Stephanie A. Wootton[2], and William Gibbons[2], *[1]Dept. of Biology and Microbiology, South Dakota State University, USA; [2]South Dakota State University, USA*

As world population continue to rise, the capability to feed those populations becomes increasingly difficult. To confront these challenges, numerous strategies have been applied in the food production system. An example of this is the addition of antibiotics to the diets of food animals in order to improve gut health and development. However, recurrent use of antibiotics prompts a predictable increase in antibiotic-resistant microorganisms. This then fuels a need to investigate possible alternatives to antibiotics (feed additives). Many feed additives are found to be inconsistent concerning inclusive health and growth of food animals. Hence, there is still a need to develop effective, environmentally safe, and natural antibiotic alternatives. Plants produce a class of antimicrobial materials known as phytoalexins. Glyceollin is the name for soybean-derived phytoalexins with three prominent isomers (glyceollin I, II, and III). Glyceollins naturally collect in soybean seeds in response to microbial (typically fungal) infection, but, glyceollin titers are at low levels and vary among soybean varieties. Therefore, in this study our goal is to maximize glyceollin production in soybeans combining fungal metabolism with various processing parameters and to compare among several soybean varieties. In a preceding study, our research team at South Dakota State University identified Trichoderma reesei NRRL 3653 as the best performing strain, stimulating the highest total glyceollin yield among many other tested strains. Hence, in this study, several processing parameters such as seed soaking time, inoculation method, incubation time, seed germination effect, seed varieties, etc. have been assessed using T. reesei.

18. **Sacha Inchi Press Cake as a Smart Ingredient for Applications in the Preparation of Functional Foods.** Luis-Felipe Gutiérrez*, *Instituto de Ciencia y Tecnología de Alimentos - Universidad Nacional de Colombia Sede Bogotá, Colombia*

Functional foods are gaining importance within the foods market, because the consumers are more conscientious about the relationship between the food consumption and health. Sacha Inchi (Plukenetia volubilis L.) is a plant of the Euphorbiaceae family with a great economic expansion in Central and South America, and in some South East Asiatic Countries. Its fruits are shell-covered kernels, considered as good source of oil (35-60%), protein (25-30%), essential amino acids, minerals, and vitamin E. They are mainly used for producing oil of high nutritional value, rich in polyunsaturated fatty acids (α-linolenic (C18:3n-3, 50%) and linoleic (C18:2n6, 35%)). The industry of Sacha Inchi oil has shown a significant growth in the last five years, as a response of the market evolution of functional foods and nutraceuticals. The main byproducts of the oil extraction process are the shell and the press-cake. These byproducts are normally used as animal feed, or discarded without any further use. Consequently, studies focused on their valorization are of quite interest. In this study we have used the Sacha Inchi press-cake (SIPC) as a smart ingredient for the preparation of various foods with functional characteristics.

Cakes, cookies and dairy products (fermented milks and fresh cheese) were elaborated employing different amounts of SIPC. In all products the fatty acid profile was improved, and the obtained products could be target as rich in omega-3 fatty acids. Moreover, the protein content was significantly increased, and the amino acid profile was enhanced. All products displayed satisfactory sensory acceptance by untrained panelists.

19. **Recent Advances in Proteins Derived Bionanocomposites for Food Packaging Applications.** Muhammad Zubair* and Aman Ullah, *University of Alberta, Canada*

This work presents a comprehensive review on the current advances in the research and development of protein-derived bionanocomposites that are used in food packaging applications. The recent growth of proteins-based material is driven by sustainability, renewability, biodegradability and low carbon footprint. The inherent drawbacks of proteins-based materials for food packaging applications are their low mechanical strength, poor thermal and barrier properties, and inferior biocompatibility. Bionanocomposites provide an opportunity to overcome issues related to physicochemical properties of the protein-derived materials. Biodegradable proteins-based bionanocomposite materials are lightweight and have high performance, which are advantageous over conventional non-biodegradable petroleum-based plastic food packaging materials. The use of biodegradable proteins-based bionanocomposite materials with high performance and light weight, making them to supersede conventional non-biodegradable petroleum-based plastic food packaging materials. So far, soy protein isolates (SPI) and wheat gluten (WG) proteins are the most studied for protein-derived bionanocomposites suitable for food packaging. Layered silicates are the most promising nanofillers used to enhance strength, improve heat resistance and better barrier properties of protein-derived materials while montmorillonites (MMT) is the mostly commonly used silicate nanofiller. This review will provide critical analysis and future perspective for the protein- derived bionanocomposite to replace petroleum-based materials for food packaging applications. Reference: This review article has been accepted for publication in "Critical Reviews in Food Science and Nutrition," Taylor and Francis Publisher.

20. **High Power Sonication of Soy Proteins in Water: Generation of Free Radicals and Free Sulfhydryls.** Md Mahfuzur Rahman****(Protein and Co-Products Division Student Travel Grant Winner)***, Bibek Byanju, and Buddhi Lamsal, *Iowa State University, USA*

High power sonication (HPS) is being researched for its ability to alter protein structure, and thus, its functionality, via intermolecular interactions. This study was conducted to quantitate different species of free radicals during HPS of soy proteins. The 5,5-dimethyl-l-pyrrolin N-oxide (DMPO) spin trap was used to capture radical species, while electron paramagnetic resonance (EPR) was used to detect, identify and quantify them. Generation of radical adducts was optimized with water and the highest concentration of radical adducts was found at 4.5 W/cm3 power density for 10 mins HPS with 500 mM of DMPO. Four protein samples in solution with added 500 mM DMPO, namely, 5% soy protein isolate (SPI), 5% isoflavonoids removed SPI (No ISO-SPI), as well as subunits 1% glycinin (11S) and 1% conglycinin (7S) were sonicated at two different power densities (~2.5, and ~4.5 W/cm3). The 5% No ISO-SPI produced higher concentration of DMPO-OH (2.14 and 1.90 µM) than SPI (1.65 and 1.71 µM) for a given power

density. For No ISO- SPI and 7s, the 4.5 W/cm3 setting produced significantly higher adduct concentration than 2.5 W/cm3; however, there was no significant difference within SPI, as well as 11S. The 11S protein produced the highest concentration of adduct (3.68 and 3.59 µM) at both power densities that is even significantly higher than water (3.43 and 2.50 µM). Therefore, HPS of soy proteins produced significant amount of hydroxyl radicals that form protein carbonyls, as well as react with free sulfhydryl content that may change soy protein's ingredient functionality.

21. **Effects of High Power Sonication on Extraction Yield and Structure of Some Plant Based Protein Isolates.** Bibek Byanju*, Md Mahfuzur Rahman, and Buddhi Lamsal, *Iowa State University, USA*

Application of high power sonication (HPS) can maximize the extraction yield of proteins and unfold the secondary and tertiary structures of proteins, resulting in altered ingredient functionality. HPS was incorporated in extraction of various leguminous proteins and its effect on structures was studied. Three legume flours, namely, defatted soy flour (particle size < 0.25mm), chick pea flour (particle size < 0.25mm), and kidney bean flour (particle size < 0.25mm), and one soy flakes 20 PDI (particle size < 0.85mm) were dispersed in distilled water (at 1:10 ratio) and sonicated at two power densities (3.0 w/cm3 and 4.5 w/cm3) for 5 min. Samples were adjusted to pH 8.5 with 2 N NaOH, stirred for 1 hr and centrifuged at 14,000 x g for 10 min. Collected supernatants were precipitated at pH 4.5 with 2 N HCl, stored at 4°C for 1 hr and centrifuged for 10 min. Precipitates were resolubilized with pH 7 DI water and freeze-dried to obtain the isolates. Protein isolates were analyzed for changes in secondary structures using SDS-PAGE and CD. All sonicated samples yielded 5% more protein isolate than unsonicated, with highest extraction yields obtained using power density 3.0 w/cm3. Soy flakes (20 PDI) showed the greatest difference in extraction (12%) between sonicated and unsonicated samples whereas chickpea showed lowest (4%). Molecular weights for sonicated and unsonicated legumes isolates were similar, indicating no peptide profile alterations by sonication. HPS treatment resulted in change in the proportion of α-helix, β-sheets and increased the proportion of unordered structure causing unfolding.

22. **Optimization of Purification Chlorogenic Acid from Sunflower Meal Co-product by Macroporous Resins: StatiC/Dynamic Study.** Tuong Thi Le*[1], Irina Ioannou[2], Armelle Ropars[3], Arnaud Aymes[4], Jean-Pol Frippiat[3], and Romain Kapel[4], *[1]Laboratoire Réactions et Génie des Procédés,Université de Lorraine,CNRS, LRGP, France; [2]LRGP - UMR CNRS 7274, France; [3]Stress, Immunity, Pathogens, Université de Lorraine, EA 7300, France; [4]Reaction and Process Engineering Laboratory UMR-7274, France*

Objectives/ Hypothesis The sunflower protein extraction purification by ultrafiltration yields large volume of effluent composed of various organic micro-solutes and chlorogenic acid (CGA). The aim of the communication was to investigate adsorption/desorption characteristics of CGA onto macroporous resins and determine optimal conditions for CGA purification on the column. Eventually, the impact of optimized CGA fraction on biological activity was investigated. Methods used Batch adsorption/desorption of CGA was studied with macroporous resins. For the dynamic study, the effect of pH (2-5) and flow rate (5-15BV/h) on productivity, recovery and CGA binding capacity were modeled by design of experiments (DoE). The effects of obtained fractions on the viability of THP-1 derived macrophages and wondered if they could moderate their LPS-induced

inflammatory response. Results XAD7, XAD16 and HP20 are effective resins among the investigated resins owing to high adsorption/desorption capacities. The adsorption followed a pseudo-second-order and Langmuir model. XAD7 has been selected for the dynamic study. The best conditions are dynamic adsorption at flow rate 15BV/h, pH 2.7 and desorption flow rate at 120BV/h, ethanol concentration at 50% (v/v). CGA obtained fractions and standard did not affect cells viability on human THP-1 derived macrophages at dose 50-100mM. TNF-α was inhibited approximately of 15-20% when we treated samples with LPS. Conclusions Adsorption of CGA is a monolayer behavior. The pH significantly affects the adsorption/desorption capacities. The obtained fractions and standard did not affect cell viability. Studies are ongoing to evaluate the capacity of these fractions to reduce THP-1-derived macrophages inflammatory response.

23. **Eucheuma (E. spinosum) powder as a partial flour replacer for gluten protein in sponge cake.** Min Huang and Hongshun Yang*, *National University of Singapore, Singapore*

Eucheuma (E. spinosum) is a seaweed that could be an alternative source for providing dietary fiber and other bioactive molecules. The effect of Eucheuma powder as partial gluten replacer (substituting 0, 5, 10, 15, and 20% of wheat flour) in sponge cake was investigated. The presence of Eucheuma changed the morphological structures of gliadins and glutenins, based on atomic force microscopy results. FTIR results indicated that intermolecular β-sheet in gliadins decreased from 16.99% (control) to 12.99% (20% wheat flour substituted by Eucheuma), while the α-helix increased from 32.59% to 38.07%. Eucheuma had a more remarkable effect on glutenins' secondary structure. The intermolecular β-sheet, α-helix, and β-sheet decreased from 26.05%, 25.54%, and 10.43% to 18.32%, 19.81%, and 2.43%, respectively. The antiparallel β-sheet and β-turn increased from 16.17% and 21.81% to 21.18% and 38.25%, respectively. This might be due to the different structures between gliadin (monomeric) and glutenin (interchain disulphide-linked polymers). Thus, the additive could not react with gliadins as easily as it did with glutenins. The mechanism of Eucheuma on the structural changes of gluten proteins was proposed. Carrageenans, the main polysaccharides contained in Eucheuma powder, are sulphated polysaccharides and strong electrolytes. During the baking process, carrageenans could bind to gliadin proteins through ionic interactions and hydrogen bonding, resulting in microstructure change. For glutenins, carrageenans could disorganize the disulphide and hydrogen links between these polymers, facilitating the unfolding of protein conformation and protein-water interactions. The changes of gluten at micro level could explain the changes of texture properties of sponge cakes. cakes.

24. **Electrospun Gelatin/Zein Nanofibers Crosslinked by Maillard Reaction for Improved Fiber Morphology Retention and Mechanical Strength.** Hui Zhang* and Lingli Deng, *Zhejiang University, China*

High power sonication (HPS) is being researched for its ability to alter protein structure, and thus, its functionality, via intermolecular interactions. This study was conducted to quantitate different species of free radicals during HPS of soy proteins. The 5,5-dimethyl-l-pyrrolin N-oxide (DMPO) spin trap was used to capture radical species, while electron paramagnetic resonance (EPR) was used to detect, identify and quantify them. Generation of radical adducts was optimized with water and the highest concentration of radical adducts was found at 4.5 W/cm3 power density for 10 mins HPS with 500 mM of DMPO.

Four protein samples in solution with added 500 mM DMPO, namely, 5% soy protein isolate (SPI), 5% isoflavonoids removed SPI (No ISO-SPI), as well as subunits 1% glycinin (11S) and 1% conglycinin (7S) were sonicated at two different power densities (~2.5, and ~4.5 W/cm3). The 5% No ISO-SPI produced higher concentration of DMPO-OH (2.14 and 1.90 µM) than SPI (1.65 and 1.71 µM) for a given power density. For No ISO- SPI and 7s, the 4.5 W/cm3 setting produced significantly higher adduct concentration than 2.5 W/cm3; however, there was no significant difference within SPI, as well as 11S. The 11S protein produced the highest concentration of adduct (3.68 and 3.59 µM) at both power densities that is even significantly higher than water (3.43 and 2.50 µM). Therefore, HPS of soy proteins produced significant amount of hydroxyl radicals that form protein carbonyls, as well as react with free sulfhydryl content that may change soy protein's ingredient functionality.

25. **A Study of Hydrolysis of Rapeseed Albumin: Kinetics Modelling and Functionalities Characterization.** Sophie Beaubier*[1], Melody Basselin[2], Xavier Framboisier[3], Olivier Galet[4], and Romain Kapel[3], *[1]LRGP - UMR CNRS 7274, France; [2]LRGP - UMR 7274, France; [3]Reaction and Process Engineering Laboratory UMR-7274, France; [4]Avril Group, France*

Rapeseed albumin (RA) has been suggested as an alternative to animal proteins for food applications. RA presents a balanced amino acid profile and notable functional properties but poor digestibility. Enzymatic proteolysis has been shown to enhance both protein functional properties and digestibility. But in general, functional properties improvements are observed at low hydrolysis degree (DH) while digestibility improvements are obtained at higher DH. The goal of this study was to determine the hydrolysis conditions [pH, temperature and enzyme/substrate (E/S) ratio] of RA using Alcalase 2.4L to obtain the best trade-off in terms of functional properties and digestibility gain. DH 18 % was highlighted. At this DH value, the protein was three times more hydrolyzed after in vitro digestion and the hydrolysate showed almost 50 % of emulsifying capacity and 220 % of foaming capacity. The second part of the study was the optimization of the operating conditions to achieve this DH by searching the best compromises between enzymatic cost and reaction time. A genetic-evolutionary algorithm was implemented to generate the Pareto front and domain presenting the targeted compromises. At DH 18 %, the Pareto front showed that the reaction would be extended by 4 h to use half of the protease amount and the optimal operating conditions were: pH 7, 59 °C and E/S between 1/15 and 1/150. This study highlighted the applicable operating conditions to improve the digestibility of RA while maintaining its techno-functional properties and minimizing reaction costs.