Protein and Co-Products Interest Area
Technical Program Abstracts

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PCP 1: Protein Co-Products: New Sources, New Technology, and New Applications

Chairs: K. Liu, USDA, ARS, USA; and H. Wang, Iowa State University, USA

The ICM Vision for Value-added Co-products in Fuel Ethanol Plants. D.B. Rivers, ICM, Inc., USA.

Historically, dry grind corn ethanol plants have produced a primary product, fuel ethanol, and a single co-product, distillers dried grains with solubles (DDGS). More recently, the majority of these plants have added the capability to recover distillers oil from the thin stillage of these plants, and thus adding value to the process. Some plants also capture carbon dioxide for sale into local carbonated beverage markets. With the advent of new technologies such as Fiber Separation Technology™, these plants now have the opportunity to transform their operations into a true biorefinery. With this in mind, ICM will present a potential pathway for the addition of multiple protein, oil, and fermentation chemical value-added co-products in the future as new technology is developed.

Sorghum Kafirin Proteins: An Overview of Their Properties and Potential Uses in Bio-based Products. S.R. Bean, USDA, ARS, USA.

Sorghum is a drought and heat tolerant crop important in areas of the central plains. In 2013, sorghum worldwide production was approximately 61 million metric tons with the United States producing about 10 million metric tons. Total grain protein in sorghum typically ranges from 7 to 16% with the major proteins being the prolamins, commonly known as kafirins. Kafirins are storage proteins and comprise 50-70% of total grain protein. Kafirins tend to be more hydrophobic than most other grain prolamins and have unique cross-linking properties when wet-cooked. These unique properties have been shown to be useful in the production of a number of bio-based products including adhesives, films and coatings, microparticles, and biomedical materials. However, there are challenges to the use of sorghum proteins in bio-based products including lack of suitable protein rich sources to isolate proteins from, the need for organic solvents and reducing agents in extracting and isolating kafirins, and effects of denaturation on protein structures. This presentation will review the properties of sorghum proteins, potential uses for bio-based products, and challenges that need to be addressed.

Effects of Steam Distillation on Extraction, Composition, and Functional Properties of Coriander (*Coriandrum sativum* L.) Proteins. M.P. Hojilla-Evangelista and R.L. Evangelista, USDA, ARS, NCAUR, USA.

Coriander (*Coriandrum sativum* L.) is a summer annual plant commonly used as fresh green herb, spice, or for its essential oil. A newly-developed process combined steam distillation and mechanical pressing to recover the essential oil and edible oil, respectively, from dehulled coriander seeds. The current work determined the impact of the dual oil extraction approach on coriander protein extractability, composition, and functional properties. Coriander protein isolates were produced by the acid precipitation method. All the dehulled samples produced protein isolates with markedly higher protein content (80-89% db) than did ground whole seed (69% db). Protein isolates showed similar amino acid compositions. Steam distillation had detrimental effects on the protein, based on major changes in SDS-PAGE band patterns and reduced protein recovery [from 40% (control) to 26%]; however, protein solubilities in steam-distilled samples were enhanced at pH 7 and 10 (90% vs. 79% in non-steam-distilled coriander).

Value Addition to Canola Meal—Progresses on Developing Canola Protein Based Wood Adhesives. N.P. Bandara and J. Wu, Dept. of Agricultural, Food, & Nutritional Science, University of Alberta, Canada.

Natural adhesives have been used for centuries before petroleum-based adhesives dominate the market due to their affordable cost and satisfactory performance. However, concerns over emission of volatile organic compounds, and non-renewability, have regained the interest of developing bio-based adhesives from renewable resources. Our previous research showed that canola adhesive prepared by conjugation of canola protein with poly(glycidyl methacrylate, GMA) showed dry, wet, and soaked strength of 8.2, 3.8, and 7.1MPa, respectively at 81.9% degree of grafting, compared favourably to soy protein adhesives. Similar to other protein adhesives, canola protein adhesive also exerts poor functionality and water resistance. Therefore, there is a need to further improve water resistance and adhesive property of canola protein. The objectives of the presentation are to review recent research and development on canola protein adhesives and to discuss the opportunities and challenges in bringing this innovation into the market. In comparison to soy proteins, canola proteins are not traditionally used for human consumption. Canola is the second most abundant oilseed in the world. Canola meal after oil processing contains ~35% protein but with limited uses except in animal feed industry. Finding alternative application of canola meal is critical to the industry to add more value to this commodity.

Composition, Mineral Profiles, and Characterization of the Ash Component for 12 Algae Samples. K. Liu, R. Barrows, and M. Woolman, USDA, ARS, USA.

Algae have been used as food, feed, fertilizer, and lately as biomass for renewable energy. Key advantages of algae include prolific growth rates, the ability to grow on lands that are marginal for other agricultural purposes, and the ability to clean up water resources with excess nutrients. Unfortunately, algal is known for high ash content. It is important to measure ash content and characterize its

chemical nature, since such properties can significantly affect the value and potential applications of the algal biomass. In the present study, 12 algae samples of different species or sources were measured for proximate composition and mineral profile, and underwent wet digestion and microscopic examination. Results showed that these algae samples varied greatly in proximate composition, with protein ranging 12.27-69.68%, oil 0.81-22.29%, ash 1.91-39.75% and total carbohydrate 21.60-75.32%, all on dry matter basis. When the algae samples were subjected to wet digestion, some portion of ash was indigestible. There was a strong positive correlation between ash content and indigestible ash content (with R2=0.9371), whereas the latter correlated positively with contents of Ca, Fe, Al, Ba, and Cr in the samples. Based on chemical data and micrographs, it is concluded that a significant portion of ash in algae was sand, particularly for those with higher ash content.

Camelina Protein-knowledge for Co-product Development from a New Industrial Crucifer Oilseed. S.P. Perera[1,2], T.C. McIntosh[1], R.T. Tyler[2], D. Hegedus[1,2], and J.P.D. Wanasundara[1,2], [1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Canada, [2]Dept. of Food & Bioproduct Sciences, University of Saskatchewan, Canada.

Camelina sativa (L.) Crantz, commonly known as false flax, is an emerging industrial oilseed crop in Canada. De-oiled meal of camelina is a rich source of protein for feed formulation and protein rich product development. However, limited information is available on camelina protein to date. Therefore, camelina seed proteins were investigated in detail to fill the existing knowledge and information gap. Camelina meal contained high levels of sulphur-containing amino acids. The polypeptide profile confirmed presence of major storage proteins cruciferin and napin. Cruciferin purification process resulted 100% protein (%N×6.25). The purified cruciferin composed of 98.4% cruciferin, 1.1% vicilin. The LC-MS/MS result confirmed expression of 11 cruciferin encoding genes and 7 vicilin encoding genes. Two different napin purification processes (meal protein extraction at pH 8.5 & at pH 3) employed resulted 100% protein (%N×6.25) and showed 4 genes that express napin. The pH 8.5 extraction provided more pure napin (99.3% napin) than that of pH 3 extraction (82.6%). FT-IR and CD spectroscopy confirmed high β-sheet structure of cruciferin and helical structure of napin. The secondary and tertiary structure of cruciferin greatly influenced by medium pH where as napin was less influenced.

Edible Insects as Sources of Proteins for Nutrition and Health. C.C. Udenigwe, H. Fisher, and C. Cutler, Dalhousie University, Canada.

Insects have been part of the human diet and recently explored for nutritional purposes with the looming global food insecurity. Edible insects contain a diversity of proteins that range in amounts from 9-75 g/100g dry weight, depending on the species. These values compare favorably with the protein contents of conventional sources e.g. soybeans and other emerging sources e.g. microalgae. Moreover, the amino acid content of insect proteins varies across the different species. Notably, *Tenebrio molitor* proteins contain higher levels of essential amino acids including leucine, tryptophan and lysine compared to some commonly used animal- and plant-derived food proteins. Despite the prospects, much is yet to be determined about the functionality of insect proteins in food matrices compared to conventional food proteins. In addition to their use in human nutrition, bioinformatics analysis of edible insect proteins showed their competitiveness for use as precursors of bioactive peptides for functional food purposes. Regardless of the economic and environmental benefits, the proteins found in a variety of edible insects provide promising high quality ingredients for the formulation of food, nutritional and health products for human consumption.

Isolation and Functionality Evaluation of Egg Yolk Granule. Z. Zhang[1], G. Cavender[1], C. Xu[1], R. Flores[1], and Y. Zhang*[2], [1]Food Processing Center, University of Nebraska-Lincoln, USA, [2]Dept. of Food Science & Technology, University of Nebraska-Lincoln, USA.

Isolation and functionality evaluation of egg yolk granule.

PCP 2a: Protein Allergenicity

Chairs: B.P. Lamsal, Iowa State University, USA; and L. L'Hocine, Agriculture & Agri-Food Canada, Canada

Effects of Food Processing on Tree Nut Allergen Immunoreactivity. S.K. Sathe, C. Liu, and V.D. Zaffran, Florida State University, USA.

Immunoglobulin E (IgE)-mediated food allergies are rising. Since there is no available cure yet, the safe way for preventing adverse reactions in sensitive individuals is to avoid the offending allergen. Therefore, efforts to reduce or eliminate food allergens by processing continue. Food processing induced protein- denaturation, modification, matrix interaction, and/or solubility change; may result in an increased, decreased, or unchanged immunoreactivity. Protein denaturation can disrupt conformational epitopes, expose hidden epitopes, or form new epitopes, which have been observed as the individual variation in sensitivity to raw and processed food allergens. With the exception of oral allergy syndrome, conformational epitopes have often been viewed as less critical compared to food allergies attributed to linear epitopes. However, a human IgE binding conformational epitope on amandin, the major allergen in almond seeds, has been recently demonstrated to be stable towards food processing. Assessing conformational epitope stability towards food processing is therefore important. In this presentation, we will discuss immunoreactivity of select tree nut allergens as affected by food processing and matrix effects.

Food Allergen Detection and Complex Foodstuffs. P.E. Johnson, University of Nebraska, Lincoln, USA.

Food allergens represent a particular set of problems for the analytical industry. Not only are food allergen proteins, with inherently diverse chemistry, physical structures and physical properties, the molecules within allergenic foods are at best poorly defined. Legislation calls for the labelling of particular species or groups of species, but analytical methods detect molecules. Particular molecules must therefore be chosen to represent species or groups and the presence of allergenic foods inferred from the presence of these molecules. In simple cases this approach can work well. However, in cases where foods are heavily processed or where components are separated, the analyte molecules chosen may cease to adequately represent the allergenic food to be detected.

We will discuss the issues surrounding the analysis of allergen in foods and heavily processed food ingredients with respect to the two most commonly used methods of analysis: ELISA and PCR. We will also address the relatively novel (for allergen analysis) technique of protein mass spectrometry and examine cases in which this technique may be able to help decipher the allergenic contents of complex and processed foods.

Tree Nut Detection and Quantification Using Monoclonal Antibody (mAb)-based Enzyme-linked Immunosorbent Assays (ELISA). C. Liu, V.D. Zaffran, S. Gupta, and S.K. Sathe, Florida State University, USA.

Tree nut-induced anaphylaxis and death have been documented. In the US tree nut allergy affects approximately 0.6% of the population. As sensitive consumers' safety depends on the avoidance of the offending allergen, reliable tree nut allergen detection methods are needed. Several enzyme-linked immunosorbent assays (ELISAs) are currently available for tree nut protein detection. The assay performance is dependent on antibody specificity, target antigen stability, and food matrix interference. Monoclonal antibody (mAb) based assays are often preferred for antigen detection due to assay specificity and reliable, continuous, and affordable supply of the detection antibody. This presentation will discuss a number of recently developed mAb-based ELISAs for detection and quantification of select almond, cashew, hazelnut, pecan, pistachio, and walnut allergens. Key assay performance elements including assay specificity, sensitivity, robustness, recovery, reproducibility, accuracy, and uncertainty will be addressed and the challenges of mAb-based ELISAs will be discussed.

Role of Food Processing and Food Matrix in Defining Protein Allergenic Potential. L. L'Hocine, Agriculture & Agri-Food Canada, Canada.

At present, it is very difficult to establish general rules concerning the effects of processing methods on the allergenic potential of foods, as these effects are highly variable and dependent on various factors, including the inherent characteristics of the protein, type, and severity of the treatment applied, the composition and structure of the food matrix (eg. the presence of other ingredients [sugars, lipids, polyphenols, etc.]). Much of our understanding on the allergenic potential of food proteins has been obtained from the study of isolated food proteins without regard to the complexities of processing operations food matrix interactions. While this approach is beneficial in understanding allergenic properties of specific proteins and proteins ingredients, it is somewhat restricted in predicting performance in real foods. Moreover, it is now well accepted that the ability of a food protein to survive the gastrointestinal passage in a structurally intact form can increase its potential to act as allergen. This presentation focuses on integrating the effects of processing and food matrix interactions on the fate of allergenic proteins in the gastro intestinal tract and how that affects their release, uptake, and their subsequent exposure to the gut immune system using in vitro models. Increased understanding of these aspects is central to food allergen risk management and will guide food processors in the development of best

practices processing/manufacturing to master and reduce the allergen risk.

Immunoreactivity of Select Rosaceae Seed Proteins.
V.D. Zaffran, C. Liu, S. Gupta, and S.K. Sathe, Florida State University, USA.

The major storage protein and allergen, amandin, is predominant in almond genotypes, related *Prunus* species, and interspecies hybrids. The objective of this study was to analyze the immunoreactivity of amandin in select Rosaceae seeds. Forty-seven Rosaceae seeds were ground to pass through a 20-mesh sieve. Full fat flours were extracted in borate saline buffer (BSB, 0.1M H_3BO_3, 0.025 M $Na_2B_4O_7$, 0.075M NaCl, pH 8.45). Solubilized proteins were determined by the Bradford method. Amandin immunoreactivity was analyzed using enzyme-linked immunosorbent assay (ELISA) and Western blot with murine anti-amandin monoclonal antibody 4C10 as the detection antibody. In ELISA, the ratio (R) = signal by the soluble proteins in the sample/signal by the soluble proteins in the reference (Nonpareil almond protein extract). 4C10 did not recognize black cherry, chokecherry, apple, and loquat seed proteins in ELISA and Western blot. The R values varied significantly ($P \leq 0.05$) with ranges of 0.54-1.19, 0.11-0.39, 0.22-0.25, 0.19-0.35, 0.06-0.32, 0.18-0.32, 0.17-0.22, 0.09-0.21, and 0.21-0.71 for almonds, wild almonds, peaches, wild peaches, plums, cherries, apricots, prunes, and interspecies hybrids, respectively. The Western blot and ELISA results were in agreement. In conclusion, under the test conditions, the selected Rosaceae species exhibited a large variation in amandin immunoreactivity.

PCP 2b: General Protein and Co-Products

Chairs: B.P. Lamsal, Iowa State University, USA; and L. L'Hocine, Agriculture & Agri-Food Canada, Canada

A Comparative Study of the Structural and Functional Properties of Flaxseed (*Linum usitatissimum*) Albumin and Globulin Fractions. I.D. Nwachukwu[1,2] and R.E. Aluko[1,2], [1]Dept. of Human Nutritional Sciences, University of Manitoba, Canada, [2]Richardson Centre for Functional Foods & Nutraceuticals, University of Manitoba, Canada.

The structural and functional characteristics of two major flaxseed proteins, namely the water-soluble albumin (ALB) and the salt-soluble globulin (GLB), were determined. Using a solution of 0.5M NaCl, flaxseed protein meal was dialyzed against water to obtain the ALB and GLB fractions. Amino acid analysis indicated that sulfur-containing amino acids (cysteine and methionine) were limiting in both proteins, while acidic and hydrophobic amino acid levels were very high. Both protein fractions were subsequently subjected to structural and functional tests. ALB showed a higher fluorescence intensity at pH 3, 7, and 9 compared to the emission spectra at pH 5, which could be as a result of the proximity of this pH point to the protein's isoelectric point. Conversely, GLB has the least fluorescence intensity at pH 3. In general, the fluorescence intensity of GLB did not vary as rapidly with changing pH as that of ALB. Additionally, surface hydrophobicity assay revealed that the GLB fraction has a higher degree of folded structure compared to the ALB. Further work to elucidate additional structural (gel electrophoresis and circular dichroism), and functional (protein solubility, foaming, and emulsion) properties of the proteins are continuing.

***In vitro* Antioxidant Properties of African Giant Land Snail (*Archachatina marginata*) Protein Hydrolysates and Membrane Ultrafiltration Peptide Fractions.** A.T. Girgih[1,2], I.D. Nwachukwu[1], M.I. Iwar[1,2], T.N. Fagbemi[1,3], and R.E. Aluko*[1], [1]University of Manitoba, Canada, [2]University of Agriculture, Nigeria, [3]Federal University of Technology, Nigeria.

African giant land snail protein hydrolysates (SnPH) were produced through enzymatic hydrolysis of defatted snail protein meal (SnPM) using a combination of pepsin and pancreatin enzymes to mimic human *in vivo* digestion of proteins. The resultant SnPH were then fractionated by membrane ultrafiltration to give peptide sizes of 1, 1-3, 3-5, 5-10, and 10kDa, which were then assayed for *in vitro* antioxidant properties. The results showed that SnPH peptides exhibited similar DPPH scavenging abilities (47-51%) which were lower than that of GSH (64%). Fractionation of SnPH did not improve superoxide radical scavenging activities (SRSA), however, it enhanced their hydroxyl radical scavenging activities. The low molecular weight (LMW) snail peptides-1 and 3kDa showed higher (2.25 and 1.44nm respectively) ferric reducing power (FRAP) activities than the SnPH (0.56nm). In contrast, the HMW snail peptides (5 and 10kDa) had superior (74 and 73% respectively) metal chelating activities (MCA), which were significantly ($p<0.05$) higher than that of GSH (64%). This work showed that snail peptides have the potential to scavenge free radicals, reduce and chelate metal ions that may promote lipid oxidation and tissue damage. Snail peptides could therefore be used as food antioxidants to retard free radical mediated morbidities.

Flaxseed Orbitides as FRET Sensor. P.D. Jadhav[1], J. Shen[1], R. Sammynaiken[2], and M.J.T. Reaney[1,3], [1]Dept. of Plant Sciences, University of Saskatchewan, Canada, [2]Saskatchewan Structural Sciences Centre, University of Saskatchewan, Canada, [3]Prairie Tide Chemicals Inc., Canada.

Flaxseed contains cyclic peptides (orbitides), linked via an N-to-C terminal peptide bond, composed of eight to ten proteinogenic amino acids having molecular weights of approximately 1kDa. Flaxseed orbitides are known immunosuppressants and induce apoptosis in human epithelial cancer cell lines. Site-selective cyclic peptide modification, through methionine, using click type reactions generates stable orbitide analogs. We have synthesized orbitide dye conjugates with coumarin antennae using this approach. Coumarin orbitide complexes also bind Eu3+ yielding compounds that transfer energy absorbed through coumarin antennae and emit photons at lanthanide wavelengths. The method has a broad substrate scope, allowing construction of peptide-based hybrid materials with useful optical, diagnostic, and therapeutic functions. The molecules tagged with fluorescent labels provide a mechanism for tracking, quantifying, and visualizing molecular distribution and movement of molecules in living cells. These fluorescent conjugates might have potential applications as biomedical agents and nanoscale materials.

Production of Microbial Protein Concentrate and 1,3-Propanediol by Wheat Thin Stillage Fermentation. K. Ratanapariyanuch[1], Y.Y. Shim[2], S. Emami[2], and M.J.T. Reaney[2,3], [1]Dept. of Food & Bioproduct Sciences, University of Saskatchewan, Canada, [2]Dept. of Plant Sciences, University of Saskatchewan, Canada, [3]Guangdong Saskatchewan Oilseed (GUSTO) Joint Lab., Dept. of Food Science & Engineering, Jinan University, China.

Fermentation with yeast produces ethanol and wheat-based thin stillage (W-TS). A second fermentation of W-TS, a two-stage fermentation (TSF) of wheat, with endemic bacteria at 25°C decreased glycerol and lactic acid concentrations while 1,3-propanediol (1,3-PD) and acetic acid accumulated. Increasing fermentation temperature to 37°C increased 1,3-PD and acetic acid accumulation markedly when compared with fermentation at 25°C. During anaerobic TSF, W-TS colloids coagulated to produce slurry and largely clarified liquid phases. The slurry floated to the top of the

fermentation medium. The endemic bacteria, present in W-TS, were largely members of *Lactobacillus panis*, *L. gallinarum*, and *L. helveticus* and this makeup did not change substantially as fermentation progressed. Furthermore, genome sequence data indicated that *L. panis* PM1B possessed sequences involved in exopolysaccharides (EPSs) production. The presence of carbon dioxide bubbles and EPS may contribute to particle-liquid separation. As fermentation media were exhausted of nutrients, floating particles precipitated. Protein contents of slurry and liquid increased and decreased, respectively, as TSF progressed. The liquid phase was easily processed by ultrafiltration without membrane fouling. These results demonstrated that TSF is a novel method for W-TS clarification.

PCP 3: Protein Interactions in Food Systems

Chairs: N.S. Hettiarachchy, University of Arkansas, USA; R.E. Aluko, University of Manitoba, Canada; and J.P.D. Wanasundara, Agriculture & Agri-Food Canada, Canada

Lipid Co-oxidation of Proteins: One Mechanism Does Not Fit All Foods. K.M. Schaich, Rutgers University, USA.

Oxidizing lipids cause significant damage to proteins, including crosslinking and scission, browning, and surface modifications that alter solubility, structural organization, and intermolecular associations. Early research naively assumed that all types of damage occurred in all food proteins. Research now shows that co-oxidation patterns vary considerably depending on the food matrix and protein composition. In tortilla chips with zeins high in sulfur amino acids, free radical-mediated disulfide and peptide crosslinking forms very large open protein aggregates; surface modifications that interfere with dye binding are also present. In contrast, in stored wheat flour, oxidation of sulfhydryl groups prevents crosslinking and interferes with gluten associations. In peanut butter, extensive modification of surface residues alters protein solubility, matrix structure, and dye-binding. Marked rearrangement of glycinin and conglycinin quarternary structure occurs with little protein scission or crosslinking. Lipid-protein interactions cause textural hardening, browning, and apparent restructuring of lipids. These results show that lipid co-oxidation of protein plays critical differential roles in oxidative degradation of foods, and that lipid concentration, amino acid composition, and nature of food matrix all appear to be important in directing specific molecular changes involved in lipid-protein co-oxidations.

Plant Protein-polysaccharide Complexes for Improved Functionality in Food Systems. L. Chen, M. Japar, C. Yang, and Z. Tian, University of Alberta, Canada.

In recent years, proteins derived from plant sources are becoming one of the food industry's fastest-growing and most-innovative ingredient segments owing to health, religious, and cost reasons. This presentation will show how plant protein functionality can be improved by adding polysaccharides through modulation of interactions between these two kinds of biopolymers. The protein samples include cereal and pulse proteins and the polysaccharides are guar gum, xanthan gum, and pectin, representing non-ionic and anionic polysaccharides with different molecular weights. We will demonstrate how polysaccharide structure (molecular weight and surface charge) impacts protein surface properties (surface tension, dilatational, and shear rheology) and network microstructures, and subsequently foaming and gelling functionality. Their potential applications in food area will be discussed.

Maillard-induced Glycation of Whey Protein: Effect on Molecular Configuration, Solubility, and Thermal Stability. B.M. Ismail, University of Minnesota, USA.

While proteins have multiple functionality and physiological benefits making them attractive ingredients in many formulations, processing imparts some challenges pertaining to thermal stability, aggregation during storage, Maillard advanced products, and sensory quality, to name a few. Understanding the molecular interactions of protein ingredients, in systems such as beverages, would aid in choosing the most suitable protein ingredient and processing conditions for a particular application. This presentation will outline the effect of Maillard-induced glycation on molecular configuration, solubility and thermal stability of whey protein for beverage applications.

Formation, Stability, and Application of Pulse Protein-stabilized Nanoemulsions. S. Ghosh, M. Yerramilli, A. Duchek, M. Primozic, and M. Nickerson, University of Saskatchewan, Canada.

The aim of this research was to utilize pea (PPI) and lentil protein isolate (LPI) in the development of nanoemulsions. PPI (5-10wt%) was used to partially replace sodium caseinate (SC) at neutral pH, while LPI solutions (0.5-5wt%) at pH3 were pre-treated with ultrasonication and homogenization followed by high-pressure emulsification to develop 5wt% oil-in-water nanoemulsions. Samples were analyzed by droplet size, zeta potential, microscopy and rheology. Accelerated shelf-life study by a photocentrifuge was used to estimate long-term stability of the nanoemulsions. While nanoemulsions stabilized with only SC and PPI displayed rapid creaming due to depletion flocculation and formed thick viscoelastic gel due to protein-droplet aggregation, respectively, mixed-protein nanoemulsions remained stable for more than 6 months. It was hypothesized that during homogenization PPI disintegrates followed by hydrophobic interactions with SC leading to the formation of soluble complex, which not only aided in emulsification, but also prevented both the destabilization mechanisms seen with individual proteins. For LPI, disintegration by pre-treatments significantly improved nanoemulsion formation and stability, although microscopy revealed extensive droplet flocculation. The mechanisms of stabilization were investigated using intrinsic fluorescence and surface hydrophobicity of the respective protein solutions.

Effects of Isoelectric Point (pI) and Hydrophobicity of Peptides in Emulsion System.

E.Y. Park[1,2], H. Miya[2], Y. Nakamura[2], K. Matsumiya[3], Y. Matsumura[3], and K. Sato*[4], [1]Dept. of Food Science & Technology, Korea Christian University, South Korea, [2]Dept. of Food Sciences & Nutritional Health, Kyoto Prefectural University, Japan, [3]Div. of Agronomy & Horticultural Science, Kyoto University, Japan, [4]Div. of Applied Biosciences, Kyoto University, Japan.

The objective of the present study was to investigate the effects of isoelectric point and hydrophobicity of protein hydrolysates on stability of oil-in-water emulsion. Wheat gluten, soy protein, and casein hydrolysates (WGH, SPH, CH) were fractionated on the basis of amphoteric nature of sample peptides by preparative isoelectric focusing. Also, the protein hydrolysates fractionated using column chromatography with amberchrom resins. Corn oil-in-water emulsion was prepared by mixing 5% (w/w) corn oil with 0.2%-1% (w/w) protein hydrolysate fractions. The efficacy of different fractions for scavenging DPPH and OH radicals, ORAC, chelating prooxidative metal ions and TBARS was investigated. Emulsion stability was examined by measuring of particle size, distribution, ζ-potential, cream layer.

Fractions from amberchrom chromatography had higher activity to prevent lipid oxidation on emulsion than autofocusing fractions. Hydrophobic fractions exhibited higher ability to emulsify to oil droplets than hydrophilic fractions. Especially, hydrophobic fraction of WGH (WGH4) had the highest emulsion stability. Distribution of isoelectric point of WGH4 fraction had different patterns compared to the others. These results suggest that hydrophobicity and isoelectric point of protein hydrolysates might be important factors influencing stability of oil-in-water emulsion.

Metal-binding to Linusorb Orbitides.

Y. Zuo[1,2], Y.Y. Shim[2,3], P.D. Jadhav*[2], J. Shen[2], N. Zhang[1], Y. Wang[1], and M.J.T. Reaney[2,3], [1]Guangdong Saskatchewan Oilseed (GUSTO) Joint Laboratory, Dept. of Food Science & Engineering, Jinan University, China, [2]Dept. of Plant Sciences, University of Saskatchewan, Canada, [3]Prairie Tide Chemicals Inc., Canada.

Linusorbs (LOs) are 8 to 10 amino acid orbitides linked *via* N-to-C terminal peptide bonds, with molecular masses of 1kDa. The cyclic LO structure makes them candidates for metal binding. Four metal salts including $Zn(OAc)_2$, $ZnSO_4$, $Pb(OAc)_2$, and $Cd(NO_3)_2$ were dissolved in solution with pure LOs. ^{1}H-NMR spectrometry and mass spectrometry (MS) were employed to detect the presence of metal-LO complexes and indicate stoichiometry and structure. Solutions were prepared with LOs that varied in methionine oxidation state to determine the impact of methionine oxidation on interactions with metal in solution. In LOs with MetO groups the ^{1}H-methyl signals of the $SOCH_3$ singlet were shifted downfield and the alpha proton of Phe^6 (4.6ppm) was altered while an amide proton at 7.7ppm disappeared. The methyl peak of methionine $MetO_2$ in related LOs does not show the same shift in presence of $Zn(OAc)_2$ and $Pd(OAc)_2$ observed in their MetO analogs. Based on NMR and MS data results, it is confirmed that metal binding strength varied by metal in the order $Zn(OAc)_2$<$ZnSO_4$<$Cd(NO_3)_2$. Singly charged [LOs+M–2H]+ and doubly charged [LOs+M–2H]2+, [2LOs+M]2+ were observed in methanolic solutions of orbitides and metals. $Cd(NO_3)_2$ exhibited stronger binding at 10–2M when compared with other metals and $Zn(OAc)_2$ the least. Metal complexes were observed forming at 10–2M to 10–4M but not at lower concentrations (10–5M to 10–8M).

PCP 4: Bioactive Proteins and Peptides: Advanced Functionalities

Chairs: H.R. Ibrahim, Kagoshima University, Japan; and H. Kumagai, Nihon University, Japan

Bioactivities of Gelatin Hydrolysates Derived from Skin of Two Fish Species. S. Karnjanapratum[2], T. Sae-leaw[2], Y.C. O'Callaghan[1], S. Benjakul[2], and N.M. O'Brien*[1], [1]School of Food & Nutritional Sciences, University College Cork, Ireland, [2]Dept. of Food Technology, Prince of Songkla University, Thailand.

Antioxidant, immunomodulatory, and antiproliferative effects of gelatin hydrolysates (GHs) prepared from (a) seabass skin and (b) unicorn leatherjacket skin were investigated in cell culture model systems. GHs were prepared from seabass skins using different processes and enzyme concentrations. GH from unicorn leatherjacket skin was prepared in a process using partially purified glycyl endopeptidase. The ability of the hydrolysates to protect against H2O2-induced DNA damage was assessed in U937 cells by the Comet assay. Both the seabass and leatherjacket skin GHs protected against H2O2-induced DNA damage in U937 cells. All samples showed immunomodulatory potential by significantly ($p<0.05$) reducing interleukin-6 (IL-6) and IL-1ß production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Antiproliferative activities of seabass skin hydrolysates were measured using human colon cancer (Caco-2) and liver cancer (HepG2) cell lines. The inhibition of cell proliferation of Caco-2 and HepG2 cancer cells occurred in a dose-dependent manner. Cell proliferation in Caco-2 cells was significantly reduced in a dose-dependent manner following incubation with leatherjacket skin GH. These results indicate that GHs from fish skin have several bioactivities which support their potential as promising functional ingredients.

Effects of ß-conglycinin on Blood Pressure and Lipid Metabolism in the Spontaneously Hypertensive Rat (SHR). K. Koba[1], K. Kawabeta[1], T. Noda[1], N. Tateiwa[1], S. Tamaru[1,2], and M. Sugano[3], [1]University of Nagasaki, Siebold, Japan, [2]Fukuoka Inst. of Technology, Japan, [3]Kyushu University, Japan.

Feeding of ß-conglycinin, one of the major components of soy protein, has been shown to decrease body fat mass, and serum and liver triglyceride levels in rats, together with an increase of serum adiponectin concentration. In our previous study, feeding of ß-conglycinin increased insulin sensitivity in rats and mice. In the present study, we examined whether it could affect blood pressure in spontaneously hypertensive rats (SHRs). Male SHRs (6 week-old) were fed the AIN-93G diets containing 20% protein; either casein (CAS), or CAS replaced with soy protein isolate (SOY), or ß-conglycinin (CON) at the proportion of 50% for 7 weeks. During the feeding period, tail-cuff blood pressure was measured every other week. Dietary SOY and more so CON as compared with CAS significantly suppressed both of systolic and diastolic blood pressures at week 4 and thereafter. The antihypertensive effect of CON (and SOY) could be partly associated with an increase of plasma adiponectin concentration. Liver triglyceride concentration significantly decreased by feeding the SOY and CON diets as compared with the CAS diet. The protein-dependent decrease could be due to a decrease of fatty acid synthesis in the liver. These results indicated that CON affected not only lipid metabolism but also blood pressure in rats, suggesting that CON has more potential to prevent metabolic syndrome than SOY.

Design of Oligo-peptides for Intestinal Absorption Model. T. Matsui, Kyushu University, Japan.

It still remains unclear whether bioactive oligo-peptides showing *in vivo* physiological effects can be absorbed as intact form through intestinal membrane. This is due to the lack of appropriate models for intestinal absorption evaluation. In this study, we attempted to design transport models for oligo-peptides (tri-, tetra-, and penta-peptides) in terms of protease resistance, on the basis of Gly-sarcosine (Sar) as a template. Among synthesized oligo-peptides, Gly-Sar-Sar was found to be an appropriate transport model for tri-peptides due to no degradation by intestinal proteases across Sprague-Dawley rat intestinal membrane, while Gly-Gly-Sar was degraded to Gly-Sar during 60 min-transport. Based on Gly-Sar-Sar as a template, Gly-Sar-Sar-Sar and Gly-Sar-Sar-Sar-Sar were successfully designed for transport models of tetra- and penta-peptides, respectively. Caco-2 cell monolayer transport experiments demonstrated that the designed oligo-peptides showed a lower transport ability by a factor of 1/10-, 1/25-, and 1/50-fold, respectively, for Gly-Sar-Sar, Gly-Sar-Sar-Sar, and Gly-Sar-Sar-Sar-Sar, compared to Gly-Sar. The cell experiments also provided useful information that the designed tri-peptide, as well as Gly-Sar, was transported across Caco-2 cell monolayers *via* PepT1, whereas the tetra- and penta-peptides were transported through paracellular tight-junction pathway.

Insight into Therapeutic Applications of Eggshell Membranes. T. Ahmed, C. Cordeiro, and M.T. Hincke*, University of Ottawa, Canada.

The innate immune protection of the avian egg is essential for food safety of the unfertilized egg and underscores the importance of the egg contents for identification of novel antimicrobials. The eggshell membranes (ESM) and associated shell participate in embryonic development by providing physical and chemical protection against pathogen invasion. We performed quantitative proteomic analysis of ESM proteins on multiple days during the three phases of embryonic development. Changes in ESM proteins that occurred during incubation revealed that protease inhibitors were present at all phases

of chick development. A group of proteins involved in calcium binding and oxygen transport were only present during the second phase. Extracellular matrix, cell adhesion proteins related to the vascularization of chorioallantoic membrane, antimicrobial proteins, and proteins involved in the binding and transport of lipids were found in the second and third phases of development. These findings provide insight into the functionality and evolving nature of ESM associated proteins involved in chick embryonic development. We have characterized the ESM proteome in order to understand functional mechanisms and gain insight into therapeutic and nutraceutical applications. The results of this study will guide further efforts to exploit the bioactive elements of the ESM for therapeutic applications.

Exhaustive Analysis of a Novel Bile Acid Binding Peptide Derived from Soybean Protein and Efficient Modification of Soystatin by Peptide Array. S. Nagaoka, Gifu University, Japan.

It is well known that soybean beta-conglycinin exhibits anti-hypercholesterolemic action. However, anti-hypercholesterolemic peptide derived from soybean beta-conglycinin is unknown. Thus, we designed to identify an active peptide which have a bile acid-binding ability from soybean beta-conglycinin. We used the peptide array to evaluate the bile acid-binding ability of peptide derived from soybean beta-conglycinin. In the peptide array, bile acid-binding ability was evaluated by the binding ability of taurocholic acid and fixed peptides on cellulose membrane, finally the antibody-taurocholic acid complexes were detected by the second antibody. We found new bile acid binding peptides in this screening. Especially, VVFLASVS had significantly lower micellar solubility of cholesterol than did other synthesized peptides *in vitro* like cholestyramine as a positive control. In rats, intestinal cholesterol absorption was significantly decreased by the administration of VVFLASVS. Thus, we found that VVFLASVS significantly decreased micellar solubility and inhibited cholesterol absorption in rats. Furthermore, we evaluated the efficient modification of VAWWMY (soystatin) activity by using the peptide array. We found PWWWMY or VIWWFK had significantly decreased micellar cholesterol solubility and inhibited cholesterol absorption in rats.

Peptides Derived from Rice Proteins Stimulate GLP-1 Secretion and Suppress Blood Glucose Elevation. H. Hara[1], Y. Ishikawa[1], M. Kadowaki[2], and T. Hira[1], [1]Hokkaido University, Japan, [2]Niigata University, Japan.

Rice includes several storage proteins, glutelin, a-globulin, and prolamin, which improve lipid and glucose metabolism. However, their mechanisms are not fully clarified. We examined effects of rice peptides on glucagon-like peptide (GLP)-1, an anti-diabetic gut hormone released from L-cells in the lower intestine by luminal stimulation of nutrients. Isolated rice proteins and their digestive products (peptides) were administered to male rats (7-10 weeks) for IPGTT (interaperitoneal glucose tolerance test). GLP-1 secretion was observed in ligated ileal loops of anesthetized rats and cultured enteroendocrine cell line, GLUTag. Administration of rice proteins or peptides suppressed plasma glucose elevation in IPGTT, and increased insulin levels in conscious rats. Portal blood concentrations of total GLP-1 and ratio of active/total GLP-1 were largely increased after ingestion of rice peptides. Rice peptides also stimulated GLP-1 secretion in GLUTag cells. These results demonstrate that rice peptides promote GLP-1 secretion by direct stimulation on enteroendocrine L-cells, and may suppress inactivation of GLP-1 by inhibition of DPP-IV activity. In conclusion, peptide derived from rice proteins improved glucose tolerance via enhancement of GLP-1 secretion. The rice peptides may be a promising food ingredient for prevention and treatment of diabetes.

Hen Egg Ovotransferrin-capped Gold Nanoparticles: A Novel Drug-targeting Strategy Against Infections Disease. H.R. Ibrahim, Kagoshima University, Japan.

Antibiotic resistance and infectious diseases are the foremost stressing clinical problems that continue to challenge the healthcare sectors. Advances in treatment of infectious diseases include development of carriers to allow targeted therapeutics that are more specific in their activity. Gold nanoparticles (GNPs) have the possibility of providing endless opportunities in the area of drug delivery due to their less toxicity, ease of functionalization, and show improved permeability and accumulation of therapeutics at the target site. GNPs are functionalized by conjugation with proteins to introduce a platform for drug loading and targeting when protein is recognized by cellular receptors. Ovotransferrin (OTf) of egg albumen is recognized by transferrin receptor (TfR) in cellular iron-uptake event. The majority of infected human cells and pathogens seem to overexpress TfR, hence OTf displays unique features worth using as drug-targeting molecules in GNPs therapy. Here, we introduce a new approach in which OTf was used as a capping ligand for GNPs in which hydrophobic antibiotics were loaded. The OTf-capped GNPs rendered antibiotics water soluble, exhibited potent antibacterial action and specific drug targeting to intracellular infections.

Structure and Content of Food-derived Soy Peptides in Rat and Human Bloods. K. Sato[1,2] and E.Y. Park[2], [1]Kyoto University, Japan, [2]Kyoto Prefectural University, Japan.

Oral ingestion of enzymatic hydrolysates of soy protein (EHSP) exerts some beneficial activities. However, the contents of the "active" peptide identified by *in vitro* assays are extensively lower than the contents necessary for *in vitro* activities, which can be arisen by degradation of the peptides in food during digestion. The objectives of the present study were to identify the indigestible peptides in EHSP and detect them in human and animal bloods. EHSP was digested with

carboxypeptidase and leucineamino peptidase. The peptides in the digest were first resolved by size exclusion chromatography (SEC). Peptides in the SEC fraction were derivatized with phenylisothiocyanate and the derivatives were resolved by RP-HPLC. The pyroglutamyl peptides were digested with pyroglutamate-aminopeptidase before derivatization with PITC. DP, DQ, DP, RP, PP, LP, IP, FP, pEL, and pEEL were identified as indigestible peptide. The indigestible peptides in the ethanol-soluble fraction of plasma were determined by LC-MS/MS in MRM mode. These indigestible peptides were detected even before ingestion. pEL was contained in highest value (>200nM) after the ingestion. It has been demonstrated that pEL shows moderation of hepatitis, colitis, and anti-anxiety activity by animal models, which suggest that at least partially pEL plays significant role in exerting biological activities by ingestion of EHSP.

Suppression of Blood-ethanol Elevation by Compounds Produced from Dipeptide and Amino Acid in Shiitake Mushrooms. H. Kumagai, S. Hironaka, and M. Akao, Dept. of Chemistry & Life Science, Nihon University, Japan.

The flavor components of shiitake mushrooms are cyclic sulfuric compounds, which are formed from a dipeptide, lentinic acid, by enzymes. When shiitake mushrooms are cut or crushed, lentinic acid is hydrolyzed to an amino acid, desglutamyllentinic acid, by γ-glutamyl transferase, and desglutamyllentinic acid is converted into sulfuric acid and pyruvate by C-S lyase. On the other hand, alcohol is metabolized to acetic acid by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) that require NAD^+ as a coenzyme. Since pyruvate is known to promote alcohol metabolism by providing with NAD^+, shiitake-mushroom extracts rich in pyruvate may also enhance the metabolism of alcohol. The present study aimed to examine the suppressive effect of shiitake-mushroom extract on the elevation of blood-ethanol level and its mechanism of action by measuring the activities of alcohol-metabolizing enzymes in liver and ethanol-absorption rate in gastrointestinal tract. Shiitake-mushroom extract significantly suppressed the elevation of ethanol and acetaldehyde concentrations in blood, and enhanced the activities of ADH and ALDH. In addition, the elevation of ethanol concentration in the blood was suppressed when ethanol was injected into the stomach or small intestine together with the shiitake-mushroom extract.

Deamidated Gliadin Induces Oral Tolerance and Prevents Cutaneous Sensitization to HCl-treated Wheat Protein. N. Matsukaze[1], R. Abe[1], M. Akao[1], H. Kumagai[2], and H. Kumagai[1], [1]Dept. of Chemistry of Life Science, Nihon University, Japan, [2]Dept. of Food Science & Nutrition, Kyoritsu Women's University, Japan.

Hyposensitization therapy using native allergens has been used to treat allergies. However, this type of therapy still harbors drawbacks, such as side effects. In our previous study, the deamidated gliadin (DG) was confirmed to have low allergenicity in vivo. Recently, it was reported that a new type of wheat allergy was developed by the use of facial soap containing HCl-treated wheat protein. In this study, we examined if DG would induce oral tolerance without causing any side effects, and if DG would prevent cutaneous sensitization by HCl-treated wheat protein.

In the first experiment, after mice were sensitized with untreated gliadin (UG) by intraperitoneal injection, DG was orally administered every other day for four weeks, and UG was orally challenged to induce systemic anaphylaxis. In the second experiment, after DG was orally administered to mice every other day for two weeks, mice were epicutaneously sensitized with HCl-treated gliadin for two weeks, and UG was orally challenged to induce systemic anaphylaxis.

Oral administration of DG to UG-sensitized mice suppressed allergic reactions and induced oral tolerance. Oral administration of DG prevented epicutaneous sensitization to HCl-treated gliadin. The results demonstrated that DG would be useful for treatment and prevention of wheat allergy.

PCP 5: Protein Processing and Involved Technologies

This session is sponsored in part by DuPont Nutrition & Health.

Chairs: C.C. Udenigwe, Dalhousie University, Canada; and N.P. Bandara, University of Alberta, Canada

Superior Functionality of Hemp Seed Protein Isolate Achieved Through Defatted Meal Carbohydrate Digestion Coupled with Membrane Ultrafiltration Processing.
R.E. Aluko, University of Manitoba, Canada.

This work aimed to optimize production of a highly soluble hemp seed protein concentrate through carbohydrase digestion of a 37% protein content industrial hemp seed protein meal (HPM). The digested fragments were removed by membrane ultrafiltration to leave behind a high-protein (74%) concentrate (mHPC). Functional properties of mHPC was compared with a commercial hemp seed protein concentrate (cHPC, 70% protein) and a traditional protein isolate produced by isoelectric pH precipitation (iHPI, 84% protein). The mHPC had significantly ($P<0.05$) higher protein digestibility (89%) when compared to ~85% obtained for the HPM, iHPI, and cHPC. The mHPC was significantly ($P<0.05$) more soluble in the pH 3-9 range, especially at pH 4.0 where solubility was 76% in comparison to the HPM and iHPI with 7.5% and 0.35%, respectively. The mHPC also had significantly ($P<0.05$) higher foaming capacity (55-98%) when compared to 10-70% for the other protein products. However, mHPC and cHPC formed poorer emulsions with bigger oil droplet sizes (4.5-15.5µm) when compared to <1µm for HPM and iHPI emulsions. The improved functional properties of mHPC suggest it could serve as a novel plant-based ingredient to formulate food products, especially those that require high protein solubility at acidic pH values.

Influence of Peptide Molecular Weight Distribution on Their Encapsulation in Liposomes. A. Mohan and C.C. Udenigwe, Dalhousie University, Canada.

Encapsulation of protein hydrolysates and peptides can facilitate the delivery of bioactive peptides. Therefore, it is paramount to determine how peptide properties (e.g. molecular weight, MW) affect encapsulation considering the molecular heterogeneity of protein hydrolysates. In this study, encapsulation of lower (<1kDa) and higher MW (5-10 and >10kDa) whey peptide fractions resulted in higher encapsulation efficiency (EE) of 94.06±0.41, 92.71±0.95 and 91.49±2.87%, respectively, compared to liposomes from intermediate MW (1-3 & 3-5kDa) fractions, which had lower EE. Although stable encapsulation products were obtained with the fractions, the encapsulated <1kDa fraction and empty liposomes had the highest and lowest zeta-potential, respectively indicating that the peptides in the suspension contributed in stabilizing the liposome. Moreover, liposomes containing the intermediate MW fraction had the highest polydispersity index (PDI, 0.59±0.03), suggesting a diversity in the type of peptides within the fraction. Low PDI observed for the higher MW fractions reflected more homogenous peptide mixtures. Furthermore, the 3-5 and >10kDa fractions yielded capsules with the highest (188.8±24.6nm) and lowest mean particle sizes (126.6±21.2 nm), respectively. Understanding the role of peptide size in encapsulation will enhance the design of efficient delivery systems for the bioactive compounds

Isolation and Identification of Protein Associated with Flaxseed Gum (*Linum usitatissimum* L.) and Its Contribution to Emulsification Properties. J. Liu[1], Y.Y. Shim[2], A.G. Poth[3], and M.J.T. Reaney[2,4], [1]Dept. of Food & Bioproduct Sciences, University of Saskatchewan, Canada, [2]Dept. of Plant Sciences, University of Saskatchewan, Canada, [3]Div. of Chemistry & Structural Biology, Inst. for Molecular Bioscience, University of Queensland, Australia, [4]Guangdong Saskatchewan Oilseed Joint Lab., Dept. of Food Science & Engineering, Jinan University, China.

Flaxseed gum (FG) is a mixture of natural polysaccharide and protein derived from flaxseed (*Linum usitatissimum* L.) that has potential for thickening foods, stabilizing emulsions, and gelling solutions. The composition and identity of protein in FG have never been reported. In this study, gum prepared from whole flaxseed was deglycosylated by treating with trifluoromethanesulfonic acid (TFMS). The resultant proteins were separated by 2D-gel electrophoresis. The major protein spots with estimated molecular weights (MW) of 10–11 kDa and 11–12 kDa were excised, digested with trypsin, and analyzed using matrix-assisted laser ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Peptide MS of tryptic digestion fragments was compared to MS of peptides from gene models available through National Center for Biotechnology Information (NCBI) database. Fragments consistent with the seed storage protein conlinin, the low-molecular-mass 2S storage flaxseed protein, were identified as the major spot constituents. Emulsification properties of FG were determined before and after protease hydrolysis with emulsion activity index (EAI) and emulsion stability (ES) as indicators. Both EAI and ES decreased from 98.7±5.4 to 59.9±3.2m^2/g and from 66.4±1.1 to 42.1±2.0%, respectively, after protease treatment.

Amino Acid Profiles of 44 Soybean Lines and ACE-I Inhibitory Activities of Peptide Fractions from Selected Lines.
N.S. Hettiarachchy, S. Rayaprolu, R. Horax, E. Satchithanandam, P. Chen, and A. Mauromoustakos, University of Arkansas, USA.

Soybean is an abundant source of protein which is known for its high nutritional value and excellent nutraceutical

properties. Forty four soybean lines were evaluated for their moisture, protein, and amino acid contents. Gastro-intestine (GI) resistant peptide fractions from 3 selected lines, R95-1705 (high in protein), and N98-4445A and S03-543CR (high in oleic acid and protein), were evaluated for their anti-hypertensive activity. Moisture, protein, and amino acid contents were determined by AACC, Kjeldahl, and AOAC methods, respectively. Proteins were isolated and enzymatically hydrolyzed with alcalase. The hydrolysates was treated with simulated gastric and intestinal juices to obtain GI resistant hydrolysates, which were then separated using ultrafiltration membranes with molecular cut-offs of <5, 5-10, and 10-50kDa. These fractions were tested for angiotensin-I-converting enzyme (ACE-I) inhibitory activity. The moisture and protein contents ranged between 5.2-12.9% and 40-53%, respectively. The amino acid analysis showed high methionine and cysteine content in R05-4494 and R05-5491 lines (high protein and fatty acid), and CRR05-188 line (high oleic acid), respectively. The 5-10kDa fraction showed highest ACE-I inhibition by 49%.The results from this study will promote high oleic soybeans as a source of protein and peptides with nutraceutical benefits.

Efficiency of Viscozyme and Cellulase in the Extraction of Proteins from Oat Brans. A. Tsopmo, O. Shituu, and R. Esfandi, Food Science & Nutrition, Carleton University, Canada.

The objective of this study was to investigate the efficiently of polysaccharide degrading enzymes in the extraction of proteins from oat bran samples. It is important to break or disrupt cell walls and thereby facilitate proteins solubilisation and extraction. To achieve alkaline conditions, salts, sonication, and high-pressure homogenisation have been used. Cereals are also high in fibers that can interfere with proteins extractions and to overcome that oat brans were pre-treated with two carbohydrases. It was found that extraction in the presence of viscozyme provide the highest amount of protein concentrate 4.97% compared to 2.89% for bran treated with cellulase and 2.1% extraction in the absence of carbohydrase. Lowry assay showed that there was no change in protein content of control and cellulase treated brans while the content of viscozyme treated brans had much higher (68%). In addition, proteins from viscozyme pre-treated barns displayed the strongest pereoxyl radical scavenging activities. Details of experimentation, molecular weight distribution and functionalities of these proteins will be presented.

PCP-P: Protein and Co-Products Poster Session

Chairs: M.P. Hojilla-Evangelista, USDA, ARS, NCAUR, USA; and P.X. Qi, USDA, ARS, ERRC, USA

1. **Profiling of Polypeptides Extracted from Water and Alkali Soluble Cottonseed Preparations.** Z. He[1] and D. Zhang[2], [1]USDA, ARS, SRRC, USA, [2]USDA, ARS, AAHRU, USA.

Cottonseed proteins and other residual fractions have shown great potential as value-added industrial products and bio-active functional materials. In this work, water-soluble cottonseed proteins (CSPw) and alkali-soluble cottonseed proteins (CSPa) were sequentially extracted from defatted cottonseed meal. Proteins of the two fractions were separated by 4-20% gradient polyacrylamide gel electrophoresis; individual bands were then excised from the gel and subjected to mass spectrometric analysis. There were total 70 polypeptides identified from the two cottonseed preparations, with molecular weights ranging from 10 to 381kDa. Putative functions of these proteins include storage, transcription/translation, synthesis, energy metabolism, antimicrobial activity, and embryogenesis. Among the most abundant are legumin A (58kDa), legumin B (59kDa), vicilin C72 (70kDa), vicilin GC72-A (71kDa), and vicilin-like antimicrobial peptides (62kDa). This work enriched the fundamental knowledge on cottonseed protein composition, and would be helpful in better understanding of the functional and physicochemical properties of cottonseed protein and for enhancing its biotechnological utilization.

2. **Ultra-structural Features of Oil and Protein Bodies of Canola and Camelina.** S.P Perera[1,2], R.T. Tyler[2], D. Hegedus[1,2], and J.P.D. Wanasundara[1,2], [1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Canada, [2]Dept. of Food & Bioproduct Sciences, University of Saskatchewan, Canada.

Canola (*Brassica napus*) and camelina (*Camelina sativa*) are crucifer oilseed crops in Canada. Oil for both food and bio-fuel applications is the high-value component of these crops. Protein-rich seed meal is the co-product of oil extraction. Increased seed oil content is a prime breeding target of these crops, and oil and protein contents show a negative relationship. Information on changing seed oil content on the oil and protein storage structures of the seed are not available. Knowledge on ultra-structural components of these oilseeds will enable to understand macro-scale changes occur in storage proteins during the oil extraction process. The effect of changes in seed oil content on oil body proteins is also not clear. A transmission electronic microscopic (TEM) investigation of ultra-structural features of cotyledon cells of canola and camelina seeds containing different oil contents was conducted. Cell size, and shape and size of protein- and oil bodies showed clear morphological differences between canola and camelina. Differences in the oil body packing density and abundance of protein bodies were observed between high and low oil containing seeds. Oil bodies covered with a protein layer were visible throughout the cells. Protein bodies showed clear crystalloid regions that are considered containing phytates.

3. **Bioinformatics and Peptidomics of Potato Protein Hydrolysates for Bioactivities.** S.R.C.K. Rajendran[1], C.C. Udenigwe[1], and B. Mason[2], [1]Dept. of Environmental Sciences, Dalhousie University, Canada, [2]Verschuren Centre for Sustainability in Energy & the Environment, Cape Breton University, Canada.

Bioinformatic tools are useful in predicting bioactive peptides from food proteins. This study focused on using bioinformatics and peptidomics to evaluate the specificity of peptide release and post-translational modifications (PTMs) in a peptic digest of potato protein isolate. Peptides in the protein hydrolysate were identified by LC-MS/MS and subsequently aligned to their parent potato proteins. Five major proteins were selected for further analysis based on protein coverage, abundance, and function. Comparison of *in silico* peptide profile generated with ExPASy PeptideCutter and experimental peptidomics data revealed several insights. The number of experimental peptic cleavage sites was found to be less compared to PeptideCutter predictions except for patatin, which had more cleavage sites than predicted. Average peptide chain length was also higher than predicted with hexapeptides as the smallest peptides identified. Moreover, PTMs of peptides, particularly Met oxidation and Glu/Asp deamidation, were also observed and these were unaccounted for *in silico*. PTMs can be attributed to aging proteins stored in the tuber, or as a result of processing conditions during protein isolation and hydrolysis with pepsin. Findings from the study provide insights on the limitations of current bioinformatic tools in bioactive peptide research, and structural modifications that can alter peptide functionality.

4. **Evaluating the Effect of Processing Conditions on the Functionality of Whey Protein Hydrolysate During Enzymatic Hydrolysis.** A. Mohan and C.C. Udenigwe, Dalhousie University, Canada.

Peptides of food-protein origin have shown potential as functional food ingredients. However, due to the reactive nature of peptides, we evaluated the effects of conditions used for enzymatic hydrolysis of protein on the fate of the reactive functionalities. Free amino contents increased resulting in 21.9 and 27.5% degree of hydrolysis with papain and Alcalase hydrolysis of whey proteins, respectively, compared to pepsin activity (3%). An increase in ferric reducing capacity was observed with peptide release during hydrolysis, but there was also remarkable 2- and 6-fold decreases in redox-active sulfhydryl (SH) groups during papain and Alcalase reactions, respectively. Furthermore, evidence for intermediate and advanced Maillard reaction products (MRPs), known antioxidative protein glycation

products, were observed during hydrolysis, and this was thought to have contributed to the hydrolysate reducing capacity, even when the peptide SH moieties were depleted. A model Maillard reaction with arginine, lactose or glucose, and reduced glutathione confirmed the consumption of SH in the presence of MRPs, and this was attributed to nucleophilic reaction with carbonyl derivatives generated during the non-enzymatic glycation reaction. The findings pose the challenge in sustaining the intact peptide structure, during processing, for specific biological properties.

5. **Antioxidative Capacity of Potato Proteins Hydrolyzed with Gastrointestinal Proteases Using *in vitro* Glutathione Oxidation Model.** M.C. Udechukwu, C. Yiridoe, A. Gibson, M. Gong, and C.C. Udenigwe, Dalhousie University, Canada.

Food protein-derived peptides can be used as natural antioxidants for promoting food quality and human health, although the fate of the peptides after oxidation remains unclear. In this study, the antioxidative capacity of potato proteins hydrolyzed with pepsin (Hp) and pepsin-pancreatin (Hppc) was evaluated using *in vitro* glutathione (GSH) oxidation model. Loss of sulphydryl groups (SH) of GSH, from 2406±36 to 787±14µM/g, was observed upon oxidation with Fenton's reagent. Both hydrolysates dose-dependently protected GSH sulphydryl from oxidation, with Hppc sparing 1,186±17µM/g at 1mg/mL. Conversely, Hppc restored two-thirds of the SH after oxidation. Owing to their high reactivity, peptides can undergo oxidative changes resulting in loss of SH, increase in free amines, and side chain carbonyl formation. Direct oxidation with Fenton's reagent resulted in decreased SH of both hydrolysates, while free amines increased in Hppc by 27%. Moreover, a decrease in the hydrolysate carbonyl groups did not support the proposed mechanism, and suggests complex reactions occurring during oxidation. Since peptides are sacrificial antioxidants, the findings revealed changes to the peptide functionality that can explain their antioxidative capacity during GSH oxidation, and offer insight on the physiological role of peptides in regulating oxidative stress.

6. **Physical Properties and Electrophoretic Characterization of Select Rosaceae Seeds.** S. Gupta, V.D. Zaffran, C. Liu, and S.K. Sathe, Florida State University, USA.

Almonds belong to the Rosaceae family which encompasses approximately 90 genera and 3000 species. Cross-breeding of different species has been used for cultivar improvement. Although globally valuable, cross-bred species remain under-investigated with respect to their protein characteristics. Therefore, the objective of the current study was to analyze the physical properties and electrophoretic characteristics of select 47 Rosaceae seeds. Seed dimensions, mass, color, and soluble proteins were determined. Soluble proteins were subjected to electrophoresis and Western blot using murine anti-amandin monoclonal antibody 4F10 as detection antibody. The length, breadth, thickness, and mass ranges were respectively 4.79-27.93mm, 3.37-15.45mm, 2.16-9.00mm, and 0.02-1.48g for shelled seeds ($P \leq 0.05$). Seed lightness (L*), redness (a*), and yellowness (b*) were respectively 20.78-62.60, 4.63-18.76, and 11.86-32.10 ($P \leq 0.05$). Total soluble protein ranged from 0.41-18.38 mg/100 mg and 2.72-26.73mg/100mg based on Bradford and Lowry protein assays, respectively ($P \leq 0.05$). Different species exhibited distinct electrophoretic profiles. Amandin was detected in all tested samples except for loquat seeds indicating the prevalence of amandin in the Rosaceae family.

7. **Protein Derived Biodegradable Food Packaging Material from Poultry By-product.** M. Zubair, J. Wu, and A. Ullah, University of Alberta, Canada.

During the last decades, petroleum derived synthetic polymers like polyethylene terephthalate, polyvinylchloride, polyethylene, polypropylene and polystyrene has extensively been used for food packaging and mostly are non-degradable. Spent fowl is the major problem for the poultry industry due to its limited disposal options and causing environmental pollution. Through current study, we have explored the possibility to transform proteins from spent fowl into green food packaging material. Proteins from spent fowl were extracted within 1 hour using pH shift method with recovery of about 74%. These isolated proteins were nano-reinforced using different nanoparticles such as layered silicates into bionanocomposite films by casting and compression molding techniques. The materials were characterized using various characterization techniques such as differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), dynamic mechanical analysis (DMA), fourier transform infrared (FTIR) and scanning electron microscopy (SEM). The nano-reinforcements with homogeneous dispersion of nanoclay lead to improved tensile strength of films. Due to addition of glycerol, the interaction between glycerol, proteins and chitosan increased and resulted better thermal properties. The results suggesting that these materials have great potential for food packaging applications.

8. **Estimation of Current Cottonseed Fiber and Seed Properties.** M.K. Dowd, S.M. Pelitire, and C.D. Delhom, SRRC, ARS, USDA, USA.

Because of the sustained efforts to breed cotton for increased fiber yield, several seed/fiber compositional properties have likely shifted. Conversations with breeders, ginners, and oil processers have identified a number of concerns in this regard, including reduced seed size, weaker hulls, greater levels of fiber contamination, and reduced kernel proteins levels—all of which affect current processing practice. To better understand these changes, a series of field cotton varieties was collected from areas around Stoneville, MS; Lubbock, TX; and Las Cruses, NM. The samples were

ginned and cleaned to determine seed-to-fiber ratios, weighed to estimate seed indexes, hand dissected to determine the proportions of linter, hull, and kernel, and analyzed for gossypol, oil, and protein. Results from the first year of study (2014) indicated that the average fiber/seed ratio (average: 1.43±0.12, range: 1.28–1.57, as is basis) has declined compared with data from the 1930s and 40s. Seed indices (average: 10.2±1.2g, range: 8.46–11.8g, as is basis) showed a similar decline. Seed proportions have changed less, although some decrease in the percentage of linters was apparent. A second year of study on an expanded number of varieties is planned.

9. **Protein Nutritional Quality, Amino Acid Profile and Digestibility of 30 Canadian Wheat Varieties.** A.J. Hernández -Alvarez, A. Hernández -Jabalera, S. Ribéreau, A. Achouri, Y. Arcand, and L. L`Hocine*, Agriculture & Agri-Food Canada, Canada.

Wheat (*Triticum spp*.) has historically played an important role in human nutrition as a dietary staple. Wheat protein quality is mainly dependent upon protein content and the balance of amino acid composition. The main objective of the present study was to evaluate protein nutritional quality of selected Canadian wheat varieties on the basis of amino acid composition and *in vitro* digestibility. The highest protein content (16.5%) was found for AC Barrie (R&D) and the lowest (12.5%) for Sampson, while the highest *in vitro* protein digestibility was found for CDC Utmost (87%), and ranged from 12.5-16.5% between other varieties. Moisture, ash and fat content varied among 7.37-10.69%, 1.51–2.07% and 1.12–1.82%, respectively. Amino acid composition showed significant variation for all genotypes. But, with the exception of tryptophan and isoleucine, major changes in amino acid composition were probably caused by environmental conditions and growing locations. The studied varieties showed high levels in aspartic acid, tyrosine, leucine and phenylalanine, and as expected, main limiting amino acids where found to be lysine and threonine. Moreover, methionine and threonine contents were the more conserved among the 30 varieties. The present study confirms the influence of environmental conditions on grain protein content as well as amino acid composition.

10. **Assessment of Protein Nutritional Quality in Canadian Soybean Varieties: Relation with Nutritional and Non-nutritional Components.** A. Hernández-Jabalera, A.J. Hernández-Alvarez, S. Ribéreau, A. Achouri, Y. Arcand, and L. L`Hocine*, Agriculture & Agri-Food Canada, Canada.

In the last decades important efforts were made through breeding and genetic modification to improve the protein quality of soybean by increasing indispensable amino acids and decreasing non-nutritional factors. However, there are important differences in the parameters used to evaluate high-quality protein varieties; in a recent effort to standardize protein scoring, FAO has recommended to replace the protein digestibility corrected amino acid score (PDCAAS) for the digestible indispensable amino acid score (DIAAS). In this study, the amino acids profiles of 26 Canadian soybean varieties were measured to compare both scores and relate them with their nutritional and non-nutritional components. Although high correlation coefficients (R>0.98) were found between the two scores, important differences in the distribution in limiting amino acid (LAA) for infants requirements among varieties were observed. When predicted with PDCAAS, Isoleucine, Leucine, Phenylalanine+Tyrosine, Lysine and Valine were found as LAA in 42.3, 15.4, 7.7, 7.7 and 26.9% of the varieties, respectively, while with DIAAS, they were LAA in 61.5, 19.2, 11.5, 3.8 and 3.8% of the varieties, respectively. On another side, study of non-nutritional factors, and other protein attributes revealed that phenolics and phytic acid contents correlated negatively with protein solubility (R=-0.754 and -0.597) and digestibility (R=-0.54, -0.50). These results highlight the importance of standardization of protein quality parameters to provide accurate information for future variety development.

11. **Effect of Novel Antioxidant Composite on the Oxidative Stability of Frying Oil and Sensory Quality of Final Fried Product.** M.A.M. Hussein, S. Saber, A. Salem, and S. Gomaa*, Innovation Center - Savola Foods Group, Egypt.

Frying process results in number of processes, including hydrolysis, oxidation and polymerization that alter the quality of frying oil and flavor stability of fried product. In this study, the effect of a novel antioxidants composite has been evaluated during deep frying. The composite is a synergetic blend of synthetic and vitamin-based antioxidants, with effective chelators and emulsifiers to obtain the optimum formula with the highest oxidative stability impact. The frying processes were carried at 180°C for French fries and sliced potato. Sensory evaluation of the fried potato has been conducted under accelerated storage conditions to determine the effect of oil oxidative stability on the shelf life of fried food. The oxidative degradation of the oil was evaluated by monitoring their respective peroxide value, free fatty acid %, content of conjugated dienes, P-anisidine value and color. Results showed that the composite has better stability against thermal oxidation when compared to TBHQ after multiple frying (P=0.05). Furthermore, sensory evaluation of the fried potatoes indicated that improving the frying oil oxidative stability has a positive impact on the sensory attributes of final product. Consequently, the proposed composite is an efficient ingredient that can improve the frying oil stability, and prolong final product self-life.