PCP 1a: Protein Nutrition and Health

Chairs: Janitha Wanasundara, Agriculture and Agri-Food Canada, Canada; Lamia L'Hocine, Agriculture and Agri-Food Canada, Canada; and Navam Hettiarachchy, University of Arkansas, USA

Overview of the Protein Quality Assessment of Quinoa (*Chenopodium quinoa*). Matthew G. Nosworthy and James D. House, *University of Manitoba, Canada*

Protein consumption is rising worldwide and there is an increasing desire for alternatives to animal-based protein from both consumers and producers. Quinoa (Chenopodium quinoa) is native to the Adean region and has been consumed in that region for centuries. It is tolerant to a wide range of environmental conditions and is notable for its nutritional properties, including protein content/ composition. The protein content of quinoa (~14%) is higher than that of cereals (~10%) or rice (~7%). In addition to protein content, however, the overall quality of the protein must also be considered. Protein quality is assessed using different methods depending upon the jurisdiction. Canada requires the use of the Protein Efficiency Ratio (PER), a growth measurement, whereas the Protein Digestibility Corrected Amino Acid Score (PDCAAS) is required in the United States. The quality of a protein is a function of overall protein content, the amino acid composition of the protein and its digestibility. This presentation will discuss the methods of determining protein quality currently in use and position quinoa as a protein source worth investigating and incorporating into novel products under development.

Functional Properties and ACE Inhibitory Activity of Mealworm Protein Isolates and Hydrolysates. Navam S. Hettiarachchy[1], Hongrui Jiang[2], and Ronny Horax[1], [1]*University of Arkansas, USA;* [2]*Institute of Light Industry and Food Engineering, Guangxi University, China*

Mealworm larva is a promising edible and sustainable high protein source. Mealworm larva protein (MP) and hydrolysates can be utilized as functional food ingredients and potential anti-hypertensive activity. The objectives were to: compare the effects of pH and sonication on MP extractability; determine the functional properties of MP isolates (MPIs) and Alcalase treated hydrolysates; and evaluate their angiotensin-I converting enzyme (ACE-I) inhibitory activities before and after gastro-intestinal (GI) environment treatment. The dispersion of defatted mealworm larvae was adjusted to pH 2.0, pH 11.0, and combined treatments (of pH 2.0 followed with pH 11.0) with and without sonication for 30 min. The solubilized MP was centrifuged, isoelectrically precipitated, and freeze-dried. Extraction at combined pHs with sonication showed the highest protein yield (47.0%) and content (77.3%). This extraction method was selected to prepare MPI to study their ACE-I inhibitory activities after alcalase hydrolysis and simulated GI juice digestion. Ultrasound treatment with the combined pH treatment significantly improved solubility from 87.3% to 93.2%, emulsion stability (22.1 to 119.4 min), foaming stability (0 to 74.2 min) (P-value <0.05), but decreased emulsifying

activity (129.9 to 94.8 m2/g) and foaming capacity (24.3 to 8.8 mL). The highest ACE-I inhibitory activity of 83.3% was observed from MP hydrolysate; however, after GI digestion the inhibition decreased to 58.0%. Protein extraction by combined acid and alkali with ultrasound treatment is a promising method to prepare MPI with improved functional properties. The MP hydrolysates have the potential as anti-hypertensive agents via their ACE-I inhibitory activity.

Mushroom Phenolics as Inhibitors of Tryptophan Oxidation and Carbonyl Formation in Bovine Proteins with Salt. Natalie G. Tom and Lilian M. Were, *Chapman University, USA*

The antioxidant capacity of dehydrated Agaricus bisporus (DAB) mushrooms against lipids in beef has been determined, however, interactions between mushroom polyphenols and bovine proteins to explain the molecular basis for mushroom's antioxidant effect has not been assessed. Oven dried or lyophilized DAB with and without 2% NaCl were added to sarcoplasmic (SP) and myofibrillar protein (MP) extracted from top round beef. The phenolic content of oven dried DAB was 2-fold higher with stronger metal chelation capacity compared to lyophilized DAB. Adding DAB in unsalted homogenates decreased volatile aldehydes by 23.08–64.29%. Adding 2% NaCl to homogenates had a pro-oxidant effect and increased volatile aldehydes by 7.69-16.67%. Carbonyl formation and tryptophan oxidation were measured spectrofluorometrically: λex=350nm, λem=400–500nm and λex=280nm, λem=300–400nm, respectively at 25°C and 37°C. The interaction between mushroom polyphenols and bovine protein were assessed using Stern-Volmer plots. There was a strong negative correlation between DAB concentration and tryptophan fluorescence in SP and MP samples ranging from –0.893 to –0.991 and –0.869 to –0.995 with added mushrooms and 2% NaCl at 25°C and 37°C, respectively, indicating no tryptophan fluorescence quenching. Tryptophan fluorescence was 100-fold higher when 2% NaCl was added to SP homogenates with DAB. As mushroom concentration increased, carbonyl fluorescence increased. However, there was no measurable carbonyls formed when 2% NaCl was added. In conclusion, 2% NaCl in SP with DAB increased volatile aldehydes which can contribute to unpleasant aromas and flavors, however, protein oxidation was inhibited, with higher tryptophan, phenolic content and strong metal chelating capacity of DAB.

PCP 1b: Advances in Bioactive Peptides

Chairs: Hitomi Kumagai, Nihon University, Japan; and Hisham Ibrahim, Kagoshima University, Japan

Occurrence of Cyclic Peptides in Human Blood after Collagen Hydrolysate Ingestion Yasutaka Shigemura*[1] and Kenji Sato[2], *[1]Tokyo Kasei University, Japan; [2]Kyoto University, Japan*

Human studies have demonstrated that ingestion of collagen hydrolysates exerts beneficial effects on skin and joint conditions. The level of hydoroxyproline (Hyp)-containing peptides increases in human blood after ingestion of collagen hydrolysates, and prolyl-hydroxyproline (Pro-Hyp) has been identified as a major component of these peptides. It has been reported that Pro-Hyp exhibits biological activities on fibroblasts and chondrocytes, indicating that it might also exert beneficial effects on human skin and joint conditions. Pro-containing peptides can form cyclic peptides, and bioactivities of food-derived cyclic peptides have been reported. Therefore, to identify new active components responsible for the beneficial effects of collagen hydrolysates, the present study aimed to detect cyclic Pro-Hyp in human blood after ingestion of collagen hydrolysate. Plasma was prepared from blood of five subjects before and after ingestion of 5 g collagen hydrolysate. Plasma was passed through a strong cation exchange resin and cyclic peptides were recovered in the unadsorbed fractions. The concentration of cyclic Pro-Hyp in plasma was determined by liquid chromatography-mass spectrometry (LC-MS) analyses. The effect of cyclic Pro-Hyp on fibroblast proliferation was examined using a cell culture system of primary cultured mouse skin fibroblasts on collagen gel. LC-MS analysis showed that cyclic Pro-Hyp level in the plasma of subjects increased after collagen hydrolysate ingestion. Cyclic Pro-Hyp enhanced primary cultured mouse skin fibroblast proliferation on collagen gel. The present study indicated that food-derived cyclic Pro-Hyp was absorbed by the human blood after ingestion of collagen hydrolysates, and cyclic Pro-Hyp may be involved in the beneficial effects of the hydrolysate on skin conditions.

Bioactive Peptides for Brain Health and its Mechanistic Exploration Shigeru Katayama*, Takakazu Mitani, and Soichiro Nakamura, *Shinshu University, Japan*

Aging is an inevitable part of life for human and is associated with declining physical and functional capacity of tissues and organs. Particularly, mild cognitive impairment and dementia has emerged as a major problem of disability in old age. There has been an increasing interest in screening potent natural dietary bioactive compounds for brain health benefits. We found that long-term feeding of soybean peptides to senescence-accelerated mouse prone 8 (SAMP8) resulted in the improvement of short-term and long-term spatial memory in the Y-maze and the Morris water maze, respectively, compared with a control group. The upregulation of neurotrophic factors such as BDNF and NT-3 and neuroprotective factors, pituitary adenylate cyclase activating polypeptide (PACAP), was observed in the brain of soybean peptides-fed group. We then tried to identify novel bioactive peptides in soybean hydrolysate which have enhancing effect of BDNF production. Some

peptides, including KGR, GRK, GR and RK increased BDNF gene expression level in mouse primary astrocytes. In particularly, the BDNF protein level in astrocytes was significantly upregulated by treatment with GR in a dose-dependent manner, suggesting that low weight molecular peptide consist of basic amino acid such as arginine and lysine in soybean protein could upregulate BDNF level. Taken together, soybean peptides would have a great potential as possible protective agents of brain aging.

Potential Bioactive Peptides from Hydrolyzed Tomato Seed Proteins Apollinaire Tsopmo* and Nasim Meshginfar, *Carleton University, Canada*

In this study response surface methodology was used to determine optimum conditions to produce an alcalase hydrolysate with the best antioxidant activity from tomato seed proteins (time 138.6 min, E/S ratio 3% and DPPH activity of 63%). Ultrafiltration membrane separation was used to concentrate the activity into the fraction with molecular weight less than 3 kDa. This fraction was further subjected to semi-preparative HPLC loaded with a C18 column. A total of seven fractions (F1-F7) were collected. F2 showed the highest DPPH radical scavenging activity while F4 better reduce phosphor-molybdate ($p < 0.05$). Both fractions were injected to the LC-ESI-MS/MS for sequencing. STTTKKHHPQYL, PSYLNTPLL, GVSLIRHVIQ and VVRPPFSQ were identified as peptides responsible for antioxidant activity, respectively. F2 and F4 were also subjected to stimulated gastrointestinal digestion to investigate possible structural changes. Comparison of HPLC chromatograms that about 90% of the peaks remained after digestion. Overall, F2 had more resistant structures than F4 under the simulated gastrointestinal digestion.

Suppression of Postprandial Hyperglycemia by Bioactive Peptides from Rice (*Oryza sativa*) Albumin Yusuke Yamaguchi[1], Shigenobu Ina[2], Aya Hamada[3], Hanae Nakamura[3], Nozomi Fujisawa[3], Makoto Akao[4], Hitoshi Kumagai[5], and Hitomi Kumagai[4], *[1]Nihon University, Japan; [2]College of Bioresource Sciences, Nihon University, Japan; [3]College of Bioresource Sciences, Nihon University; [4]Dept. of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, Japan; [5]Faculty of Home Economics, Kyoritsu Women's University, Japan*

Diabetes mellitus is a serious disease affecting over 400 million people in the world. One of the effective ways to prevent diabetes is to suppress the increase in postprandial blood glucose level. Albumin in some cereals such as wheat and buckwheat are known to inhibit α-amylase activity and retard the digestion of starch in the small intestine. At first, we hypothesized that albumin from rice might have the same function as that from wheat and buckwheat, but it did not inhibit the activity of α-amylase from mammalian. In spite of this lack of inhibitory effect on mammalian α-amylase activity, it suppressed postprandial hyperglycemia even on glucose loading. Rice albumin of 16 kDa was hydrolyzed to an indigestible high-molecular-weight peptide (HMP) of 14 kDa and low-molecular-weight peptides (LMP) by digestive enzymes. Therefore, in this presentation, the mechanism of action of rice albumin was investigated by fractionating rice albumin into HMP and LMP after hydrolysis by digestive enzymes, and administering each of them to rats together with glucose. Interesting enough, both HMP and LMP suppressed

postprandial hyperglycemia on glucose loading. The suppressive effect of HMP on hyperglycemia would be attributed to its tight structure that could adsorb small molecules like glucose. This structural rigidity prevents not only hydrolysis by digestive enzymes but also heat denaturation during processing. As rice albumin retained its high solubility in water even after heating at 100°C for 2 hours, the contribution of disulfide bonds for thermal stability of rice albumin was also examined.

PCP 2a: Proteins for Delivery Functions

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

Nature-inspired Protein Nanotechnology for Delivery of Nutraceuticals and Anti-cancer Drugs
Yoav D. Livney*, *Department of Biotechnology and Food Engineering, Technion, Israel Institute of Technology, Israel*

Humanity faces high prevalence of several non-communicable diseases, including cancer and metabolic-syndrome-related obesity, diabetes & cardiovascular diseases. To overcome these global challenges, multidisciplinary efforts must address both prevention and treatment. The enrichment of food and beverages with disease-preventing nutraceuticals is challenging due to poor solubility, sensitivity to deterioration, adverse sensory properties of certain nutraceuticals, high costs of the bioactive and of the solubilization/encapsulation materials used, regulatory hurdles including strict limitations on health claims, and on novel ingredients and technologies, religious constraints (Kosher, Halal), allergenicity of certain encapsulating materials, consumer demand for label friendly ingredients ("all natural ingredients"), poor bioavailability of certain hydrophobic nutraceuticals, and more. Chemotherapeutic drugs have numerous drawbacks e.g. poor solubility, limited oral bioavailability, poor selectivity, adverse side-effects, and limited efficacy due to drug resistance. A suitable and effective oral drug administration form would drastically improve quality of life of cancer patients and significantly reduce treatment costs by minimizing unnecessary hospitalizations, which put the patients at a life-threatening risk due to antibiotic-resistant bacteria prevalent in hospitals. Over the last decade, my research team at the Lab of Food Physical Chemistry and Biopolymeric Delivery Systems has been developing various nature-inspired protein nanovehicles for delivering health-promoting bioactive compounds for either food & beverage enrichment, or for drug delivery, with emphasis on anticancer chemotherapy. The talk will highlight the main technologies we have introduced, including: re-assembled casein micelles, thermally treated β-lg-EGCG nanoparticles, beta casein (β-CN) micelles, hydrophobin co-assemblies, potato-protein nanoparticles, and Maillard conjugates for targeted growth-support to probiotics in the microbiome.

Protein-lipid Complexes for Delivery of Nutraceutical Compounds Lingyun Chen*, Guangyu Liu, and Zhigang Tian, *University of Alberta, Canada*

Objective: Food protein and lipid-based nanoparticles have attracted recent interest as a means of delivering nutraceuticals. This research aims to develop a new protein-lipid composite nanoparticle as delivery system of hydrophilic nutraceuticals. Methods: The nanoparticles were prepared from food proteins and lipids and vitamin B12 was used as a nutrient model. Results: The complex nanoparticles demonstrated high vitamin B12 encapsulation degree of 69%. In addition, they could resist a simulated low pH, pepsin gastric environment and then subsequently was able to control the rate of vitamin B12 released into a simulated

intestinal environment. An in vitro cell evaluation demonstrated the nanoparticles are internalized into Caco-2 cells via energy-dependent endocytosis to significantly increase the uptake and transport efficiency of vitamin B12. In an in vivo study, vitamin B12 loaded nanoparticle increased serum vitamin B12 more efficiently than the free forms in rats. Conclusion: The new protein-lipid composite nanoparticle has significant potential as a basic platform for the delivery of many different hydrophilic nutraceuticals.

Design of Alginate Based Microgels for Protein Encapsulation and Delivery: pH Triggered Release Ruojie Zhang*, Zipei Zhang, and D. Julian McClements, *University of Massachusetts Amherst, USA*

Microgels are particularly promising vehicles for encapsulation and protection and release of proteins because they can be fabricated from food-grade biopolymers using mild processes. In current study, a model globular protein (whey protein) was encapsulated in microgels (D43 = 290–520 µm) fabricated using an extrusion device with a vibrating nozzle to inject alginate into calcium solution. Protein-loaded microgels were fabricated at three different pH values (pH 3, 5 and 7) to study the influence of protein-alginate electrostatic interactions on protein encapsulation, retention, and release. Protein encapsulation and retention was highest at low pH, while protein release was highest at high pH. Confocal microscopy and spectrophotometry measurements indicated that increasing the pH could trigger protein release from alginate beads formed at pH 3. These results suggest that hydrogel beads are suitable for encapsulation and pH-triggered release of proteins, which may be advantageous for certain food applications.

Hemp Protein as an Encapsulating Agent to Produce Hemp Oil Powders Anusha Samaranayaka*, Moumita Ray, and Udaya N. Wanasundara, *POS Bio-Sciences, Canada*

Many new food products containing whole hemp seed, hemp nuts, and its oil and protein fractions are finding their way into food and health-food market as a result of numerous nutritional benefits associated with protein, oil, and fiber fractions. While the demand at present is mainly for the hemp proteins, hemp oil is also high in alpha-linolenic acid (ALA) and gamma-linolenic acid (GLA) and has an ideal ratio of omega-3 to omega-6 fatty acids to be used for human nutrition. Unsaturated nature of hemp oil however makes it prone to be oxidized. Microencapsulation is a technique which is gaining much attention recently in the food industry to incorporate healthy ingredients into conventional and novel products while minimizing detrimental effects to product quality and stability. Use of vegetable proteins as microencapsulating agents reflects the “green” tendency and most are known to be less allergenic compared to animal derived proteins. Present study was conducted to assess the feasibility of using hemp proteins as a carrier to make powdered hemp oil products. Wet extraction techniques were employed to extract proteins and these proteins alone, or in combination with other carriers were used to produce oil powders with 30–50 % oil loading. Particle size, encapsulation efficiency, and oxidative stability of oil powders were assessed. Results revealed a great potential for hemp proteins to act as effective encapsulating agents,

which will increase the utilization of hemp-derived products and would add an extra value to oil powders by having a great source of protein incorporated into it.

Development of Protein-based Filled Hydrogels for Oral Delivery of Lipophilic Active Ingredients

Zipei Zhang* and D. Julian McClements,
University of Massachusetts Amherst, USA

For certain applications in the food, personal care, and pharmaceutical industries there is a need to design delivery systems to encapsulate lipophilic active agents, protect them during storage, and then release them within the mouth. Hydrogel particles fabricated from food-grade biopolymers can be used to develop this type of oral delivery system. In this study, two types of hydrogel particles were fabricated by electrostatic complexation of a protein (casein) and polysaccharide (alginate) or a protein (gelatin). Relatively small hydrogel particles were formed at pH values where electrostatic complexation was induced between casein and alginate (pH 4.5) or casein and gelatin (pH 5.8). The caseinate-coated fish oil was selected as the model lipophilic active agent, which was loaded inside hydrogel particles during their fabrication. Our stabilities studies indicated that encapsulation of the fish oil droplets inside the hydrogel particles improved their stability to lipid oxidation compared to conventional emulsions, which was attributed to a high local concentration of antioxidant protein around the emulsified lipids. Light scattering and confocal fluorescence microscopy indicated that lipid droplets encapsulated within the hydrogel particles were released under simulated oral conditions, which was triggered by a pH (casein and alginate) or temperature change (casein and gelatin). Our results suggest that hydrogel particles could be useful as oral delivery systems for lipophilic active agents.

PCP 2a: Proteins for Delivery Functions

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

Understanding Cohesive Strength from Plant and Animal Proteins Charles R. Frihart *, *Forest Products Laboratory, USA*

Though historically protein adhesives provided good wood bonds, they have been largely displaced by more moisture durable, fossil fuel derived adhesives. Recently, however, new co-adhesives or co-reactants for protein adhesives have been discovered. In addition, a better understanding of protein technology is advancing the strength attainable with these adhesives. Continuing concerns about formaldehyde emissions from urea-formaldehyde adhesives, and a desire for bio-based materials, continue to drive commercial interest in adhesives from plant proteins. Since determining that adhesion to wood is generally not an issue even under wet conditions, we have focused our research on increasing the cohesive strength of soy flours, concentrates, and isolates. Cohesive strength of soy proteins can be increased by higher bonding temperatures and hot water/steam processing of the soy. Another approach is to add co-reactants or co-adhesives to the soy material. The processes, performance, and potential mechanism for these reactions are discussed. There are opportunities for further improvement if we can learn better how to control protein structure.

Enzyme-assisted Aqueous Extraction of Soybean Oil and Protein: Focus on Solving the Wastewater Problem Xiaonan Sui* and Lianzhou Jiang, *Northeast Agricultural University, China*

Commercial oils from oil-bearing plant materials are commonly extracted with an organic solvent. Due to the increased awareness of safety and environmental issues associated with organic solvent extraction, it is necessary to develop alternative methods. Enzyme-assisted aqueous extraction processing (EAEP) has been widely investigated to extract oil and as well as proteins from many oilseeds. Yet, during EAEP of soybeans, a large aqueous fraction (also known as soy skim) is produced. It is normally considered as wastewater, and raises a disposal issue. Therefore, developing a greener way to utilize the skim fraction could promote the industrialization of EAEP method. In this updated work, extruded soybean flakes were hydrolyzed using Alcalase to separate free oil, cream, skim and residues. The skim fraction containing soybean protein hydrolysate (SPH) was then collected. The antioxidant activity of the SPH was analyzed using chemical, simulated gastrointestinal digestion and transepithelial transport methods. SPH displayed DPPH radical scavenging (IC50=4.22 mg/mL) power, ABTS•+ radical scavenging (IC50=2.93 mg/mL) power, reducing power and metal ion chelating activities (IC50=0.67 mg/mL). Furthermore, SPH significantly ($p<0.05$) inhibited the generation of intracellular reactive oxygen species (ROS) in Caco-2 cells. After simulated GI digestion, the antioxidant properties of SPH were enhanced

except for a decrease in ABTS•+ radical scavenging activity. After transepithelial transport, the permeates of GI-digested SPH maintained partial antioxidant activity and the LC-MS/MS data further identified the absorption of soybean peptides. These findings suggest that SPH from soy skim contains the antioxidant peptides that are potentially bioavailable and can therefore serve as a promising source of functional food ingredients.

An Improved Wet Method to Process Oats into Fractions Enriched with Protein, Beta-Glucan, Starch or Other Carbohydrates Keshun Liu*, *USDA, ARS, USA*

Oat is among very few grains that contain mixed linkage (1-4, 1-3) beta-D-glucan (BG), a soluble fiber having health benefits. There is a growing interest in incorporating oat or its components into the human diet. Various methods to process oats into value-added fractions have been available, but almost all focused on enrichment with only one or two nutrients while disregarding others. This presentation describes an improved wet method developed at a USDA lab to fractionate oats into several value-added ingredients, with each enriched for protein, BG, starch, or other carbohydrates, respectively. Effects of NaOH concentration and solvent to flour ratio on concentration and recovery of the four main nutrients (protein, BG, starch and other carbohydrates) were also investigated. Seeds of Lamont, a hulless oat variety, were ground into whole grain flour, defatted with hexane, and then extracted with a dilute alkaline solution. Fractions enriched for protein, BG, starch or other carbohydrates were recovered subsequently. Wet fractions were dried in a forced air oven at 60ºC before chemical analysis and mass measurement. Results show that both NaOH concentration and solvent to flour ratio had significant effects on nutrient concentration and recovery in each type of fractions. The extent of the effects varied with fraction type and nutrients. Overall, the wet method, once optimized, was effective in recovering the major nutrients from oats into their respective fractions.

Functional Properties of Mealworm Proteins
Changqi Liu[1], Emily Woolf[1], Jing Zhao[2], Sarah Kim[1], and Shruti Shertukde[1], [1]*San Diego State University, USA;* [2]*California State University, Los Angeles, USA*

Mealworms (*Tenebrio molitor*) are a potential source of high quality dietary proteins. Functionalities of mealworm proteins have not been thoroughly investigated. The objective of this study was to evaluate the hydration, hydrodynamic, and surface properties of mealworm proteins. Proximate compositions of mealworm larvae were analyzed using the AOAC official methods. Mealworm proteins were extracted in water or 0.5 M NaCl solution by homogenization (worm-to-solvent ratio = 1:10 w/v) at 30,000 rpm for 30 s. Protein solubility in the range of pH 1-13 was determined by the Bradford method. Least gelation concentration (LGC), emulsifying activity index (EAI), and emulsion stability index (ESI) of the extracted proteins were analyzed. One-way ANOVA and Fisher's least significant difference test at P^2/g) and ESI (35.2±3.4 min) than proteins extracted by the salt solution (168.8±5.8 m^2/g and 12.3±0.1 min). Both water and salt extracts had a LGC of 8 mg/mL. The results indicated that mealworm

proteins have great potentials as a functional ingredient for food formulation.

Emerging Camelina Protein: Extraction, Modification and Structural/Functional Characterization Baraem Ismail*, *University of Minnesota, USA*

By 2025, the global demand for protein ingredients is expected to reach 6.8 million tons and generate revenues of nearly $50 billion. Specifically, there is a growing interest in developing new plant-based protein ingredients that will replace the market sector or at least a portion of it that has been largely dominated by traditional protein ingredients such as dairy proteins (whey and casein) and soy proteins. Reasons that have led to this interest include increasing cost of traditional protein ingredients, rising incidences of allergenicity, increasing number of vegan and health conscious consumers, and the constant search to overcome functionality limitations of specific proteins. Other drivers include a growing interest in sustainable and environment friendly sources, valorizing by-products by utilizing current processing streams, finding a unique and a competitive place in the market, replacing unfamiliar ingredients with functional proteins (clean label), and utilizing all possible resources to expand the overall ingredients supply. Some may also argue that the food industry is aging, and there is a pressing need for innovation. 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. This presentation will cover the evaluation of Camelina protein. Camelina (Camelina sativa, a Crucifer seed and a member of the Brassicaceae family) is a sustainable oilseed crop that is high in both fat (30-38%) and protein (25-30%). Thus, it is an attractive choice for the production of both oil and protein ingredients. Two protein extraction approaches, alkaline and salt extraction, and their impact on the structural and functional properties of camelina protein will be discussed. Protein yield, content, structural characteristics, and functional properties of the produced camelina protein concentrates (CPC, 70-80% protein) and hydrolysates (CPH) will be evaluated and compared to reference proteins, whey protein isolate (WPI) and soy protein isolate (SPI).

ANA 3.1/PCP 3a: Bioprocessing for New/Value-added Protein Utilization: Digestibiity Issues/Analytical Measurements

Chairs: Sneh Bhandari, Merieux Nutrisciences, USA; Buddhi Lamsal, Iowa State University, USA; and Bishnu Karki, South Dakota State University, USA

Matrix Effect on the *in vitro* Immunodetection of Food Allergens. Qinchun Rao, Xingyi Jiang, and Behnam Keshavarz, *Florida State University, USA*

To protect the public health, the U.S. food manufacturers have been required to label food allergens or ingredients derived from eight major allergenic foods since 2006. Currently, the presence of misbranding and/or undeclared food allergenic residues is the No. 1 cause of food recalls in the US. In order to (1) fight food fraud, (2) better comply with the food regulations, (3) decrease the food recalls economic loss to the food industry, and (4) reduce the risk of food allergy, it is necessary to develop reliable and robust in vitro detection methods to prevent the occurrence of undeclared allergenic residues in foods. As the major fish allergen, parvalbumins (PV) from mullet and salmon in two sample models were used to elaborate the relationship between matrix effect, extractability of PVs, and their thermostability during in vitro immunodetection. Matrix-induced thermal instability of PV was mainly due to physical (hydrophobic effect) and chemical (thiol-disulfide interchange) interactions. Our results illustrate that the addition of sodium dodecyl sulfate (SDS, surfactant), β-mercaptoethanol (reducing agent) or ethylenediaminetetraacetic acid (EDTA, metal chelator) during sample preparation could not only increase the extractability of PV but also enhance its immunodetection using two PV-specific monoclonal antibodies. Our findings demonstrate an overdose on EDTA made PV monomer Ca2+-free and led it undetectable by PARV19. Overall, it is never enough to emphasize that matrix effect on target analyte quantification is unignorable during food allergen detection because any false negative assay outcomes may induce potential or severe life-threatening allergic reactions in consumers.

Protein Quality Evaluation in Protein Enhanced Formulations Including Those Based on Oilseed Based Proteins Sneh Bhandari*, *Merieux Nutrisciences, USA*

There's growing evidence that high-protein food choices do play a role in health and more consumers are looking for high quality proteins from varied sources including those from plants. Oilseeds protein are becoming a newly recognized source of dietary proteins particularly to meet growing needs of large segments of word population. More and more new protein products are becoming available from different oilseeds for opportunity to incorporate in a broader variety of foods to make nutritionally enhanced products. The accurate assessments of protein quantity and quality in newly available sources of the dietary proteins and the formulations and product based on those has is important. This evaluation has acquired additional importance now because of current new trends of the development of protein enhanced products. Protein quality needs to be evaluated to determine its percent daily value for nutritional labeling in US and can be done by the use of PDCAAS in food meant for ages >1 year. Formulation of a nutritionally incomplete protein

with an ingredient containing complementary protein can result in a product with complete protein with improved PDCAAS value. FAO has proposed a new protein quality measure digestible indispensable amino acid score; DIAAS which is yet to receive a wider acceptance by regulatory agencies.

Simultaneous Quantification of Hydrolysis Degree, Protein and Mean Weight of Peptides Released during Enzymatic Proteolysis. Sophie Beaubier[1], Irina Ioannou[1], Xavier Framboisier[2], Olivier Galet[3], and Romain Kapel[2], *[1]LRGP - UMR CNRS 7274, France; [2]Reaction and Process Engineering Laboratory UMR-7274, France; [3]Avril Group, France*

Enzymatic proteolysis is an industrial process used in a wide range of applications (improvement of functionalities, nutrition, bioactive peptides production…). Study of this process consists in kinetic follow-up of the protein conversion rate, the hydrolysates size and the hydrolysis degree. To determine these 3 parameters, three different analysis are required which can have drawbacks particularly for vegetable protein hydrolysis. The communication presents an original methodology to quantify simultaneously these three criteria by size-exclusion chromatography (SE-HPLC). The approach is based on absorbance profiles and the estimation of molar extinction coefficient of each point of this one from the mixture aminogram of hydrolysates. Peak area of protein eluted into column dead volume informs on protein conversion rate and the peptide signal permits to determine size and DH of hydrolysates. As a first step, the approach was tested on the hydrolysis of animal and vegetable proteins with Alcalase 2.4L. A corrective factor was determined for each substrate from the linear correlation between the DH value obtained with the methodology and TNBS method, used as reference method. Then experimental validation tests realized with others enzymes were analyzed by SE-HPLC and TNBS and pH-Stat methods. Good quantification of DH value was observed (90 % of validation tests) compared to TNBS method. The developed methodology is a powerful tool for monitoring enzymatic proteolysis both for animal and vegetable proteins while minimizing time. Moreover, it could be used for functionalities, digestibility or bioactivities analysis of produced hydrolysates.

Nutritional Evaluation of Modified Carinata Meals in Finfish. Tom Kasiga and Michael Brown, *Dept. of Natural Resource Management, South Dakota State University, USA*

The recommended inclusion of carinata Brassica carinata meal (CM) in animal feeds is currently ≤10%. However, CM use in fish feeds has not been tested but will likely be limited by high concentrations of fiber, glucosinolates (GLS) and sinapine. GLS and sinapine tolerance in Hybrid Striped Bass Morone chrysops ♀ X M. saxatilis ♂ (HSB) was tested using incremental amounts of cold-pressed carinata meal (CPCM). Inclusion of >2.71 µmoles of GLS and >0.31 mg of sinapine/g (>10% CPCM) of diet reduced feed intake, resulting in reduced fish growth. To reduce antinutrients, we processed CM by aerobic conversion (AC) using fungi ssp. followed by a single wash to produce aerobically converted carinata meal (ACCM). In a Rainbow Trout Oncorhynchus mykiss (RBT) trial, we replaced up to 75% of FM in a low animal protein (20%) diet containing FM as the only animal protein source. Replacements ≥ 50% FM (≥10% ACCM) reduced fish growth. Due to low

utilization of ACCM by RBT, we used low (20%) but similar animal protein contents (10% FM and 10% poultry by-product meal) and included up to 30% ACCM or 30% double-washed carinata meal (DWCM) in HSB diets. Growth of HSB fed the FM reference diet was similar to that of HSB fed 30% ACCM or 30% WCM. Thus, ACCM can replace more FM in diets but in combination with animal meals (≥20%). Because ACCM was low in GLS and sinapine but high in fiber, current research is focused on reducing fiber in ACCM.

PCP 3b: Bioprocessing for New/Value-added Protein Utilization: Technologies

Chairs: Buddhi Lamsal, Iowa State University, USA; and Bishnu Karki, South Dakota State University, USA

Fungal Fermentation of Rapeseed Meal for Better Animal Feed Bo Hu*, *University of Minnesota, USA*

The objective of this research is to study the various treatment procedures to enhance the nutritional value of rapeseed fiber and rapeseed meal for monogastric animals. Rapeseed meal contain high concentration of pectin-like polysaccharides, hemicelluloses, and cellulose that are resistant to degradation in the gastrointestinal tract of pigs, and their protein content is relatively low to be considered as high value animal feeds. The physiochemical (combinations of alkali, heat, and pressure) and biochemical (fungal treatment) methods of treatment are evaluated for the Rapeseed meal for its fiber degradation and protein digestibility. The results show that fungal treatment of rapeseed meal decreases the content of anti-nutrient chemicals, increases the overall content of proteins and key amino acids

Extraction and Properties of Protein from Camelina Engineered to Produce Acetyl-triacylglycerols (Camelina Acetyl-TAG). Mila P. Hojilla-Evangelista*[1], Roque L. Evangelista[1], and John Ohlrogge[2], *[1]USDA, ARS, NCAUR, USA; [2]Michigan State University, USA*

Camelina (*Camelina sativa*, Brassicaceae) has attracted interest for its seed oil as alternative feedstock for biofuels production. Researchers at Michigan State University successfully engineered camelina to produce seeds with oil containing high levels of acetyl-triacylglerol (acetyl-TAG) by incorporating the diacylglycerol acetyltransferase gene isolated from Burning Bush (*Euonymus alatus*). Acetyl-TAG has an acetyl group in the *sn*-3 position instead of the typical long-chain fatty acids found in vegetable oils. Acetyl-TAG would be beneficial as fuels, emulsifiers, plasticizers, biolubricants and hydraulic fluids. However, it is not yet known how such genetic modification impacts other components in camelina, especially the proteins, which are the likely major co-product of oil processing. This work, then, evaluated the composition, extractability and properties of protein in camelina acetyl-TAG press cake and compared them with those of wild camelina. Both ground, defatted camelina samples contained 45 % crude protein. Major proteins were the NaCl-, acetic acid-, and water-soluble fractions, which accounted for 40 % of total protein. Camelina acetyl-TAG had more of the acid- and NaOH-soluble proteins. Electrophoresis showed notably different band patterns of ethanol-, acid-, and NaOH-soluble proteins from wild and acetyl-TAG camelina. Conventional acid precipitation that we used previously for pennycress gave poor protein yields (< 5 %), although camelina acetyl-TAG produced twice as much protein extract than did wild camelina. Modifying the extraction method (e.g. removal of precipitate solubilization step) resulted in more than 15 % protein yield and improved protein purity (86 % versus 72 % crude protein) for camelina acetyl-TAG.

Oilseed Protein Based Biomimetic Adhesive Inspired by Mussel Adhesion Nandika Bandara*[1], Hongbo Zeng, and Jianping Wu[2], *[1]Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada; [2]University of Alberta, Canada*

Soy protein is extensively explored as a potential alternative to synthetic adhesives. However, the challenge remains in developing soy-derived adhesive with acceptable adhesion and water resistance. Biomimetics is considered as a promising tool in developing biobased materials. Therefore, the objective of this study was to develop mussel inspired biomimetic soy protein adhesive via converting inherent amino acid, tyrosine into 3,4-dihydroxyphenylalanine, the main factor responsible for mussel adhesion. Soy proteins were reacted with tyrosinase, and NaOH and $FeCl_3$ were added to mimic mussel adhesion mechanism. Adhesion was significantly increased from 4.97 ± 0.94 & 1.79 ± 0.52 MPa (control) to 13.21 ± 1.58 & 3.93 ± 0.21 MPa (modified adhesive) for dry and wet strength respectively. In addition, prepared adhesive showed acceptable adhesion to mica, glass, and polystyrene. The improvement in adhesion was a result of DOPA mediated polymerization and crosslinking, increased cohesive interactions, hydrophobic interactions with wood surface and effect of NaOH and Fe^{3+} in accelerating protein crosslinking.

Production of Proteins from Partially De-oiled Mustard Flour Levente L. Diosady, and Bih King Chen*, *Dept. of Chemical Engineering, University of Toronto, Canada*

Mustard proteins have a balanced amino acid profile, and effective binding and emulsion-stabilizing capacities, therefore isolated and purified mustard proteins have extensive potential applications in meat processing and in other food preparations. Earlier we developed a patented membrane-based process for protein isolation from fully defatted mustard flour. The process involves the protein extraction, chemical treatment, centrifugation, membrane separation (ultrafiltration and diafiltration), isoelectric precipitation, and drying. The process had been used previously in more than 10 pilot scale tests in various facilities to produce protein isolates from hexane-defatted mustard meal. In the present work we tested the feasibility of eliminating the solvent extraction of the oil prior to protein isolation. In bench-top experiments with the pressed meal obtained from G.S. Dunn Dry Mustard Millers all of these steps were optimized to ensure the quality and yield of the final products. The lab-scale processing yielded 2 main products: a precipitated protein concentrate with 80% protein (as is, N×6.25), and a soluble protein concentrate with 71% protein. Both had very low phenolic content (~0.15%). They were light in color, and bland in taste. These protein products can be used as emulsifiers and binders in meat processing. The meal residue left after the process contained 10% protein, and was light in color and bland in taste. The process will be adapted to pilot-plant scale to produce sufficient amount of materials for the evaluation of their functional properties under large scale plant conditions.

PCP 4: Pulse Proteins

Chairs: Tanya Der, Pulse Canada, Canada; and Chibuike Udenigwe, University of Ottawa, Canada

Global Market Trends for New Pulse Product Development Tanya Der*, *Pulse Canada, Canada*

Abstract not available.

Cropping Location and Year Effect Protein Content and Amino Acid Score of Different Lentil Varieties Matthew G. Nosworthy*[1], Jason Neufeld[1], Tom Warkentin[2], and James D. House[1], *[1]University of Manitoba, Canada; [2]Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, Canada*

Objective To determine whether there is any impact of cropping location on protein content and amino acid score (AAS) of different lentil varieties and whether there is significant inter-year variation in those factors. Methods Red/ green lentils (RL, GL) (varieties- RL: Maxim, Imax, KR-1, GL: Greenstar, Invincible, Impower) were collected from Elrose, Sutherland, Swift Current (SC) in 2012, 2013, 2014. Protein content and amino acid composition were determined using standard methods (combustion and chromatography respectively). Data were analyzed using 2-way repeated measures ANOVA, Tukey's MC test. Results In 2013 RL protein content was higher in Elrose (24.56±0.76%) and Sutherland (25.81±0.38%) compared to SC (21.38±1.23%) ($p < 0.01$) Conclusions Protein content and AAS are altered by growing location/year, with varietal selection being important to maximize nutritional benefit.

Bioaccessibility of Bioactive Compounds with Dipeptidyl Peptidase-IV and α-glucosidase Inhibitory Activities in Pulses Chibuike C. Udenigwe[1], Elisa Di Stefano*[1], and Teresa Oliviero[2], *[1]University of Ottawa, Canada; [2]Wageningen University, Netherlands*

Diabetes is a growing public health concern, expected to reach 10% of the world population by 2040. Dipeptidyl peptidase (DPP)-IV and α-glucosidase are enzymes that play a central role in post-prandial glucose regulation in humans and are established targets for antidiabetic drugs. Plant-derived DPP-IV and α-glucosidase inhibitors have been reported, mostly resulting from chemical and/or enzymatic hydrolysis of specific plant-extracted compounds (namely phenolics, peptides, triterpenoids, non-starch polysaccharides). However, although these compounds are usually consumed as part of food products, the effect of food matrix on the bioaccessibility and bioavailability of these compounds remains unclear. In this study, DPP-IV and α-glucosidase inhibitory activity of five widely consumed pulses (kidney bean, chickpea, fava bean, yellow pea, green lentil) was investigated. Whole legumes were studied in order to simulate the process occurring during food consumption and to understand the microstructure interactions occurring between pulse components. Two pre-treatments, germination, and microbial fermentation by Lactobacillus plantarum, were evaluated for their effect in disrupting the pulse microstructures and subsequent release of bioactives with DPP-IV and α-glucosidase inhibitory activities before and

after in vitro digestion. This study opens a new perspective for investigating the bioavailability of bioactive molecules provided through food products.

Functional and Sensory Characterization of Pre-treated Yellow-Eyed Beans Marcia English*, *Saint Francis Xavier University, Canada*

The nutritional content of beans make them good candidates for gluten-free applications, however the presence of off-flavours may be a limiting factor. In the present study, the impact of soaking on the functionality of bean flours and the addition of chocolate on the overall acceptability of cake style brownies made from these flours was investigated. Raw yellow-eyed beans were soaked for 24 h (pre-treated), dried in a kitchen oven and then milled using a kitchen mill. Total starch content, protein profile, water binding capacity (WBC), and water absorption index (WAI) of the bean flour samples were compared to an all-purpose flour control. The overall acceptability of cake-style brownies made with different combinations of the flour samples was also evaluated. Starch content varied significantly among the pre-treated (42.9± 3.2%, $p < 0.05$) and untreated yellow-eyed bean flour samples (35.3 ± 1.9%). Compared to the untreated sample, there was a 1.3% increase in both the WBC and WAI values of the pre-treated samples. Protein bands with molecular weight 15 kDa identified in the control were missing in the bean flour samples, whereas the 37-kDa protein band detected in the untreated sample was absent in the pre-treated sample. Brownies containing 100% pulse flours were described as nutty and beany, however, consumer acceptability of 50% treated: all-purpose blends were not significantly different from the control brownies. In conclusion, soaking yellow-eyed beans improved the WBC and the WAI properties of the corresponding flour samples and changed the protein profile. However, the use of soaking and chocolate flavour could not mask beany and nutty aromas in cake-style brownies substituted with 100% pre-treated bean flour. Future experiments will explore fermentation strategies to limit the presence of off-flavour compounds in pulse flours.

Pulse Ingredients as an Alternative to Soy in the Production of Meat Analog via High Moisture Extrusion Cooking Jenni Harrington*, *Buhler Inc., USA*

Texturized vegetable proteins are alternative to meat and produced via High Moisture Extrusion Cooking. Interest and consumption of these new products are on the rise among end consumers in North America and Europe. Currently, soy is the dominating raw material, as it is easily accessible and texturable. However, soy contains allergen and is often genetically modified, raising concerns among consumers. Pulses have great potential to replace soy in this application. The aim of this presentation to review challenges and opportunities for pulses in meat alternative application.

Oleogelation using Pulse Protein-Stabilized Foam Athira Mohanan*, Yan Ran Tang, Michael Nickerson, and Supratim Ghosh*, University of Saskatchewan, Canada*

Substitutes for trans- and saturated fats for oil structuring is an active area of research and hold significant promise in the area of food and nutrition. Present study explored the use of foams stabilized by combinations of two pulse proteins (protein concentrate and isolates from faba bean and pea) and a polysaccharide

(xanthan gum) for the structuring of canola oil. Foams were prepared using a mixture of 5% protein and different concentration of xanthan gum (0, 0.125% and 0.25%) at different pHs (3, 5, 7 and 9) and characterized by over run, foam stability, and microstructures. Oleogels were prepared by adding oil into the freeze-dried foams. Porous dried foams rapidly adsorbed the added oil through hydrophobic interactions. Oil binding and holding capacities, large and small deformation rheologies and microstructures of the oleogels were determined. Freeze-dried foams were able to hold oil about 50 times its weight. Foams stabilized by protein concentrates displayed better foam stability, oil binding, and oil holding capacity than the corresponding protein isolates. The foams prepared above pH 5 displayed better oil holding, and binding properties than the other foams. However, gel strength, and spreadability of the oleogel displayed a complex relationship with protein source, pH and polysaccharide concentration. Overall, the study has revealed that pulse protein-stabilized foams can be used for oil structuring and the properties of the oleogels can be tailored by changing the pH, amount of polysaccharide, foam stability and types of protein extracts.

Wet Fractionation of Lentil and Faba Bean for Protein Ingredient Production: Effect of Processing Factors on Ingredient Quality and Functionality Anusha Samaranayaka*[1], Rick Green[1], Michael Nickerson[2], and Shannon Hood-Niefer[3], *[1]POS Bio-Sciences, Canada; [2]University of Saskatchewan, Canada; [3]Saskatchewan Food Industry Development Centre Inc., Canada*

Lentil and faba bean are two pulse crops showing great potential to develop functional protein ingredients to address the global demand for alternate and sustainable protein sources. Various dry and wet processing techniques can be employed to produce protein concentrates and isolates using pulses. Selection of an extraction and fractionation technique as well as process factors involved such as pH, temperature, shear forces, processing time, and drying technique used can have tremendous impact on flavor, color, techno- and bio-functional properties of the protein ingredient produced. Alternatively, process factors can be manipulated in order to obtain a protein ingredient with desired purity, functionality, and/or organoleptic properties for specific food application. This presentation will describe composition, functional characteristics and potential food applications of protein concentrates and isolates produced from lentil and faba bean. Effect of processing factors on product composition, quality, and ingredient functionality will be discussed providing insights into potential protein ingredient development with target functionality.

Nanoparticles Prepared from Desolvation of Pea Protein Concentrates as a Potential Stabilizer for Pickering Emulsions Chi Diem Doan* and Supratim Ghosh, *University of Saskatchewan, Canada*

The aim of this research was to synthesize nanoparticles from pea proteins, and to exploit their potential Pickering-like stabilizing behavior in oil-in-water emulsions. The nanoparticles were prepared by diluting 5 wt% pea protein solutions (pH 9.0) in varying concentrations of ethanol (1–5 times the protein solutions) at pH 3 and 10. Higher ratios of ethanol caused a greater extent of desolvation, higher loss of hydrophilic property and larger size of particles (0.40 ± 0.02

µm at 1 time versus 579.14 ± 512.41µm at 5 times of ethanol dilution). After homogenization at 5,000 psi for 5 mins, the pea protein particles displayed a uniform size distribution, with a smaller size at pH 10.0 compared to pH 3.0. At pH 10.0, the particles prepared at higher temperatures (95oC) revealed a smaller size distribution (0.93 ± 0.15 µm) than those synthesized at 25oC (4.40 ± 0.76 µm). The protein particles were collected by centrifugation, subsequently were re-dissolved in water at 1.5 wt%, and used as the aqueous phases of 5 wt% oil-in-water emulsions prepared with high-pressure homogenization at 20,000 psi for 8 cycles. At pH 10, the protein particles displayed about 80–90% re-dispersibility and formed stable emulsion with an average droplet size of less than 0.5 µm. Nevertheless, creaming appeared in emulsions prepared at pH 3.0 due to the partial dissolution of the protein particles. This study successfully demonstrates the prospective role of pea protein nanoparticles as a Pickering stabilizer of oil-in-water emulsions.

Effect of the Carriers on the Microstructure and Functionality of Spray Dried Pea Protein Isolate
Yang Lan* and Jiajia Rao*, North Dakota State University, USA*

Pea protein as an alternative source of animal proteins in protein fortified beverage has recently attracted strong interest. However, low solubility in acidic conditions and unpleasant beany flavor limit its application in food. This study aimed to understand the effect of carriers (gum arabic (GA) or maltodextrin (MD)) on microstructure of spray dried pea protein isolate (PPI) and their functional properties (e.g., solubility and beany flavor). PPI formulated with 0, 10, 20, 30, 40 wt% of GA or MD were obtained by spray drying at 160 °C. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) were employed to elucidate microstructure of all samples. SEM results showed that interaction of PPI-GA led to more wrinkled surface with higher surface area than PPI and PPI-MD. FTIR results indicated that there was stronger H-bond interaction between PPI and carriers (gum arabic, maltodextrin). PPI showed two diffraction peaks around 8.9° and 19.5° suggesting the remaining of crystalline after extraction. Diffraction intensity at 8.9° of PPI faded gradually as the increase of both GA and MD, indicating the formation of amorphous structures. Therefore, percentage protein solubility (PPS) at pH 7 appreciably increased to 98.7% and 95.5%, respectively, as increase of GA and MD. GA was more effective to improve PPS at pH 4.5 than that of MD. Moreover, the amount of beany flavor associated volatile compounds, 1-pentanol and 1-octen-3-ol, were largely reduced in both groups. The findings have important implications for the design and utilization of PPI in food.

Reformulating Cereal-based Foods with Pulses: Effect on Nutrient Density and Environmental Sustainability
Christopher Marinangeli*, *Pulse Canada, Canada*

Interdisciplinary research across nutritional, agricultural, and environmental sciences is required to delineate how current practices and modifications to food systems will address nutritional and environmental challenges. Nutrient density and environmental sustainability often underpin messages that advocate for increasing levels of pulses in human diets. Although Canada is the largest producer of yellow peas, compared to other pulses, their

consumption in North America is relatively low. However, there is an opportunity to enhance the utilization of yellow peas in processed foods to simultaneously increase their nutritional and sustainability profiles. Using Canadian cropping data, the Nutrient Balance Concept (Fern et al. 2015), and lifecycle analysis, this study investigated the effects of reformulating bread, breakfast cereal, and pasta on nutrient density and environmental sustainability. Moreover, this study combined data corresponding nutritional and greenhouse emissions as a means to evaluate both outcomes simultaneously. Inclusion of higher amounts of pulses in food products could bring substantial nutritional and environmental advantages, in terms of lower GHG emissions, and a more nutritionally balanced diet.

PCP 5: Protein and Co-Products General Session

Chairs: Nandika Bandara, Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada; and Rotimi Aluko, University of Manitoba, Canada

Iron Release Properties of Pulse Seed Ferritin Concentrates After Simulated in vitro Gastrointestinal Tract Digestion Rotimi Aluko*, *University of Manitoba, Canada*

The aim of this work was to produce ferritin (iron-binding proteins) concentrates from pulse seed proteins and determine their in vitro iron-releasing properties when subjected to simulated gastrointestinal tract digestion. Ferritin was extracted from moon dal washed (MDW), chickpea (CP), green lentil whole (GLW), yellow split peas (YSP) and red lentil (RL) seeds. The ferritin protein was initially solubilized in phosphate buffer (pH 7.2) that contained NaCl, followed by addition of MgCl2 and sodium citrate to promote protein aggregation. The aggregated protein was collected as a precipitate after centrifugation and then freeze-dried as the ferritin concentrate. Protein content of the ferritin concentrates ranged from 56% for YSP to 70% for MDW while gross yield varied from 9.7% for MDW to 19.1% for CP. Gel electrophoresis confirmed the presence of 26.5 kDa and 28 kDa bands, which represent the two major ferritin polypeptides. Iron content was significantly ($p<0.05$) higher in YSP and GLW ferritin concentrates (~45 mg/100 g) when compared to RL (35 mg/100 g), MDW (34 mg/100 g) and CP (30 mg/100 g). During simulated gastrointestinal digestion, most of the iron was released by pepsin digestion but additional iron release occurred with subsequent pancreatin digestion. Susceptibility of the ferritin protein cage to protease digestion was confirmed by gel electrophoresis, which showed disappearance of the 2 ferritin polypeptides after enzyme digestion. The results suggest that these pulse ferritin concentrates may be useful alternatives to inorganic iron in the management of iron deficiency anemia.

Anti-inflammatory Properties of Potato Protein Hydrolysates in Primary Cells, Cell Lines and Mice Model Chibuike C. Udenigwe[1]*, Ming Gong[2], Emeka B. Okeke[3], Jude E. Uzonna[3], *[1]University of Ottawa, Canada; [2]Dalhousie University, Canada; [3]University of Manitoba, Canada*

Inflammation is a series of non-specific immune responses and irregular inflammation can lead to injury and chronic diseases such as cardiovascular diseases. Tumor necrosis factor (TNF)-α is one of the primary pro-inflammatory cytokines mediating inflammatory responses including release of reactive oxygen species. The anti-inflammatory role of dietary proteins is thought to be mediated by peptide motifs released after enzymatic hydrolysis during digestion. To test this hypothesis, potato protein hydrolysates produced with eight proteases, including gastrointestinal enzymes, were evaluated in this study for anti-inflammatory activities using mice ANA-1 and C57BL/6 mice primary cell models as well as inflammatory BALB/c mice model. The potato protein hydrolysates suppressed tumor necrosis factor (TNF)-α release and reactive oxygen species level in the cell cultures. The hydrolysates produced with ficin (HFic), containing 3,364 peptides that were identified by peptidomics, exhibited a

dosage-dependent pattern in inhibiting TNF-α release in both cell models. Intraperitoneal administration of HFic to BALB/c mice resulted in the dose-dependent reduction in the amount of cytokines including TNF-α and interleukin (IL)-6, but not IL-12, in the mice serum and peritoneal fluid. The findings indicate that HFic, which is comprised of >70% peptides with molecular size

Antioxidative Peptides from Sorghum Proteins and Composition-Activity Relationships

Yonghui Li*, *Kansas State University, USA*

Antioxidants are widely used in food, feed, and pet food industries to delay lipid oxidation and prevent quality deterioration. Many food proteins possess antioxidant 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. Kansas is producing nearly half of the U.S. grain sorghums. About one-third of the sorghum is being used for ethanol production, resulting in a large amount of distiller's grain byproduct with ~30% protein. Selection of appropriate proteases that hydrolyze protein at targeted amino acid residues and optimization of the degree of hydrolysis (DH) and hydrolysate composition via manipulating the reaction variables are vital to the overall antioxidant activity of the hydrolysate. The objectives of this study were to: 1) study the effect of various hydrolysis variables on the antioxidant activity of sorghum protein hydrolysate; 2) understand the composition-structure-activity relationships of the hydrolysates; and 3) deliver high-performance antioxidative peptides from sorghum proteins. Ten commercially available food grade proteases were evaluated. Reaction parameters, including enzyme to protein ratio, protein concentration, and reaction time were optimized in terms of protein solubility and antioxidant activity. Promising hydrolysates were further purified by membrane ultrafiltration, size exclusion chromatography, and ion exchange chromatography and analyzed to understand the composition and activity relationships. Neutrase, Flavourzyme, Bromelain, and Pepsin were promising enzymes in producing sorghum protein antioxidants. Medium size hydrolysates (3-10 kDa) were more effective antioxidants. A Strong correlation exists between peptide composition and structures and their antioxidative performances.

Greening, Reducing Capacity, and Protein Oxidation in Sunflower Butter Cookies as a Function of pH

Sihui Liang*[1], Lan Han Tran[2], and Lilian M. Were[1], *[1]Chapman University, USA; [2]Nong Lam University, Vietnam*

Sunflower butter can be a potential non-allergenic legume and tree nut alternative in the bakery industry. The greening reaction, caused by sunflower's oxidized chlorogenic acid covalently bonding with amino groups at alkaline pH, can however lower visual acceptability of sunflower butter bakery products. This study focused on using four acidic ingredients (sour cream, buttermilk, yogurt, and honey) against an alkaline control (maple syrup) to investigate the effect of pH and aw on greening, protein oxidation, Folin-Ciocalteu and ABTS scavenging capacity changes in sunflower butter cookies. The pH, aw, and chlorogenic acid-amino group conjugates (trihydroxyl benzacridine derivatives) of cookies were ranked in same order: maple syrup>sour cream≈buttermilk> yogurt>honey. pH was strongly positively correlated with Hunter

–a* greening (r=0.918) and % greening (r=0.736). Strong correlations between aw and Hunter -a* greening (r=0.946) and % greening (r =0.956) were found. Cookies made with honey had the highest soluble chlorogenic acid, protein, and Folin-Ciocalteu reducing capacity/FCRC. The FCRC was negatively correlated with pH (r=-0.974), while ABTS scavenging capacity was similar amongst cookies. Cookies made with dairy ingredients had a higher tryptophan fluorescence intensity (λe=280nm, λem=300-500nm) attributed to higher tryptophan content in the dairy ingredients. Moderate and strong negative correlation between pH and tryptophan fluorescence (r=-0.637) and pH with Schiff base formation (r=-0.963) were found. In conclusion, honey prevented post-baking greening due to its lowest pH and aw and had the highest FCRC, while acidic diary ingredients provided more tryptophan. Use of honey instead of maple syrup could thus decrease greening in sunflower butter cookies.

Transforming Soy Adhesives to Provide Greater Strength. Christopher Hunt and Charles R. Frihart*, *Forest Products Laboratory, USA*

Soy adhesives have been the most studied protein-based wood adhesives, but there are many aspects that are not clearly understood. Although groups pursuing research in this area emphasize protein denaturation can lead to improved bond performance, the literature gives almost no guidance on HOW to denature for optimized adhesive performance. Our program aims to advance the performance of protein adhesives by identifying the denatured protein's properties associated with good bonds. We will present data showing that the details of the soy alteration process are critical to generating high performance soy–based adhesives and discuss the progress that we have made in understanding what is required to coax soy into providing high strength bonds. We also discuss how soy is normally delivered denatured and show that commercial soy protein isolate has excellent bond performance relative to native protein or other treatments. We suspect that many of the lessons of soy may be applicable to adhesives based on other proteins as well.

Recovery and Utilisation of Pelagic Processing Blood-Waters from Marine Processing Plants and Utilization of Protein for Nutritional and Potential Health Applications Maria Hayes[1], John Fagan[2], Michael Cannon[2], and Michael Gallagher[2], [1]*Food BioSciences Department, Teagasc Food Research Centre, Ireland;* [2]*Bord Iascaigh Mhara, Ireland*

Processing of pelagic fish generates large volumes of blood-water at the tanker, hopper and process stage of processing. This is an economic and environmental cost for processors. However, blood-water also contains proteins, peptides and lipids with potential market applications. The aims of this work were to assess the quantity and volume of protein, lipid and small molecules present in fifty blood water samples recovered from eight different processors based in Co. Donegal at different stages of pelagic fish processing including at the tanker, hopper and process step. Blood waters were stabilised and proximate analysis carried out to determine the protein, ash, and lipid content of each. Molecular weight cut off (MWCO) filtration was used to recover proteins between 3-100kDa in size and these were screened for potential bioactivities including antioxidant, Angiotensin-I-converting enzyme inhibitory (ACE-I) and functional attributes. The

total antioxidant capacity of recovered blood-water samples was assessed based on oxidation of 2, 2'-Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) to $ABTS^{\cdot}+$ by metmyoglobin. Values ranged from 0.39 Trolox equivalents –TE (mM Trolox/mg) for blood-water recovered from horse mackerel processing to 0.97 mM TE/mg for blood-water recovered from mackerel processing at the tanker stage of processing compared to 3 mM TE/mg for reservatrol (positive control). Potential applications for recovered proteins include pet-food and animal feed.

Modelling and Optimization of Rapeseed Protein Extraction and Purification Claire Defaix*[1], Frantz Fournier[1], Arnaud Aymes[2], Olivier Galet[3], and Romain Kapel[2], *[1]LRGP - UMR CNRS 7274, France; [2]Reaction and Process Engineering Laboratory UMR-7274, France; [3]Avril Group, France*

Rapeseed is the second world largest produced oilseed behind soybean. The process to recover oil generates a protein-rich by-product, the meal. Its proteins are mainly globulins (50%) and albumins (40%) with very distinct and interesting properties for human nutrition. However, it is today's challenge to overcome the complexity of protein extraction from the meal and their purification. Indeed, antagonistic factors affect both process steps and must be understood. An original methodology was developed based upon equations from mass balances and design of experiments which are applied to multicriteria optimization. This tool rests on genetico-evolutionary algorithms and results in several sets of operating conditions, all equivalent regarding the process performances. This methodology was applied to proteins extraction and their purification by ultrafiltration. Concerning the extraction, pH, ionic strength and temperature were considered. Transmembrane pressure, velocity, temperature and membrane cut-off were taken into account for ultrafiltration step. The performance criteria focused on the yields, albumins / globulins ratio, phenol contamination, colour and phytic acid complexation. The experiments showed the highest protein yields were obtained with high pH and ionic strength but this also lead to low proportions of the desired protein. Hence, a compromise is to be found. Using multicriteria optimisation, operating conditions were chosen and scaled-up from laboratory (100 mL) to pilot scale (4 L) to produce isolates containing at least 65% of globulins and 90% of albumins. Eventually, this methodology is a powerful tool to produce rapeseed proteins, according to chosen performance criteria, at any scale.

Preparation of Highly Purified Lignan from Defatted Sesame by Supercritical Carbon Dioxide and Low-Temperature Crystallization. Heejin Kim[1], Nakyung Choi[2], No Young Kim[1], Jong Hun Choi[3], Chulyoung Lee[3], and In-Hwan Kim[1,2*], *[1]Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea; [2]Dept. of Integrated Biomedical and Life Sciences, Graduate School, Korea University, Republic of Korea; [3]R&D Center, Nongshim, Republic of Korea*

Sesame seed contains abundant lignan compound such as sesamol, sesamin and sesamolin which are widely known to have potential biological activities including antioxidant activity. In this study, highly purified lignan (lignan content of ca. 90%) was successfully produced by a combination of supercritical carbon dioxide ($SC\text{-}CO_2$) and solvent crystallization. Firstly, sesame oil with 3.5% lignan content was prepared from defatted sesame

meal by fractional SC-CO_2 extraction at 41.4 MPa and 40°C. Lignan was further enriched from the sesame oil with 3.5% lignan content by repeated fractional SC-CO_2 extraction. Under the optimum conditions, which are a temperature of 50°C, and a CO_2 flow rate of 2.5 mL/min, the lignan content was increased up to ca. 18% from 3.5%. Finally, highly purified lignan was achieved from the lignan-rich sesame oil with ca. 18% lignan content by crystallization using petroleum ether as a solvent. With an operation temperature of -20°C, the content of lignan in solid fraction was increased up to ca. 90% with a yield of 30%.

PCP-P: Protein and Co-Products Poster Session

Chairs: Mila Hojilla-Evangelista, USDA, ARS, NCAUR, USA; and Navam Hettiarachchy, University of Arkansas, USA

1. **Pilot Plant Fractionation of Canary Seeds and Functional Properties of Protein Isolates.** Allaoua Achouri[1], Delphine Martineau Côté[1], Stéphane Sirois[1], Emily Mason[1], Pierre Hucl[2], Elsayed Abdel-Aal[1], and Lamia L'Hocine*[1], *[1]Agriculture and Agri-Food Canada, Canada; [2]University of Saskatchewan, Canada*

Canary seed (Phalaris canariensis L.) is a true cereal produced primarily in Western Canada as accounting for more than 60% of the world production. The use of canary seed is currently limited to feed for caged and wild birds. Significant efforts have been made to develop novel glabrous (hairless) canary seed varieties which have been approved for human consumption by Health Canada and the U.S. Food and Drug Administration in 2015 as a new wholegrain cereal food. In the present work, four canary seeds varieties were fractionated in a pilot plant scale-up process to optimize the preparation of highly purified fractions including proteins, starch, oil and total fibers components. The protein isolation process was optimized at different pHs of extraction resulting in protein recovery ranging from 55 to 62%, purity of 89 to 93% and yields of around 12.3 to 14.9g/100g flour, for the studied varieties. The yields of other components, starch, oil and total fibers were of 47–54%, 6.6–9.4% and 12.4–20.7%, respectively. Osborne's fractionation process of canary seed defatted flour showed that proteins were mostly composed by prolamines (38%), soluble albumins (3.6%), globulins (4.3%) and glutelins (1.75%). Above 38% of the canary seed proteins were insoluble. The study of the functional properties of canary protein isolates (CPI) revealed, higher solubility at acidic than alkaline region; enhanced fat and water absorption capacities and notably higher foaming and emulsifying capacities than soy protein isolate (SPI). With growing global demand for protein, canary seed has high potential as a source of high quality and functional cereal protein.

2. **Inhibitory Activities of *Amaranthus viridis*, *Telfairia occidentalis* and *Solanum macrocarpon* Leaf Extracts Against Carbohydrate-Digesting Enzymes.** Olayinka A. Olarewaju, Adeola M. Alashi, and Rotimi Aluko, *University of Manitoba, Canada*

Objectives/Hypothesis: Inhibition of digestive enzymes such as α-glucosidase and α-amylase is a promising therapeutic strategy for the treatment and management of chronic health conditions such as obesity and diabetes. Therefore, an effective way to prevent obesity is to inhibit dietary sugar digestion within the gastrointestinal tract. The aim of this study was to characterize the inhibitory action of polyphenol-rich extracts of Amarathus viridis (AV), Solanum macrocarpon (SM) and Telfairia occidentalis (TO) leaf on α-amylase and α-glucosidase activities as well as to study the mode of enzyme inhibition.

Methods: Ground leaf powder (10 g) of SM, AV and TO were extracted twice with double distilled water at ratio 1:20 for 2 h with continuous stirring at 60°C and centrifuged at 10,000 g for 30 min. The supernatant was filtered and concentrated using a rotatory evaporator at

60°C. Extracts were screened to test their potential anti-obesity activity using α-amylase and α-glucosidase assays. The mode of the enzyme inhibition was also determined using Lineweaver-Burk plot.

Result: Our findings revealed that the extracts inhibited α-amylase (86.15, 53.19 and 52.60 % for TO, SM and AV respectively) and α-glucosidase (45.97, 37.94,20.16 % for TO, SM and AV respectively) dose-dependently with TO extracts having significantly ($P < 0.05$) higher α-amylase and α-glucosidase.

Conclusion: All the three vegetable extracts had strong in vitro carbohydrate digestion-inhibitory properties, which make them potential agents for reducing calorie intake as a preventive or treatment tool against chronic diseases such as diabetes and obesity.

3. **Inhibitory Activities of Yellow Field Pea Protein-derived Peptides Against α-amylase and α-glucosidase.** Temitola O. Awosika and Rotimi Aluko, *University of Manitoba, Canada*

Objective: To produce yellow field pea protein-derived peptides that can inhibit activities of α-amylase and α-glucosidase.

Methods: Protein hydrolysates were produced via enzymatic hydrolysis of pea protein isolate using four enzymes: alcalase, pepsin, trypsin and chymotrypsin. Each hydrolysate was fractionated into different peptide sizes (<1, 1–3, 3–5 and 5–10 kDa) by membrane ultrafiltration. The unfractionated hydrolysates and peptide fractions were then analyzed for their ability to inhibit in vitro activities of α-amylase and α-glucosidase. The mode and kinetics of enzyme inhibition were then determined using the most active peptide fractions.

Results: At the highest concentrations tested (α-amylase: 225 µg/ml and α-glucosidase: 20mg/ml), the chymotrypsin-derived peptide fraction (<1 kDa) had the highest α-glucosidase inhibitory activity of 53.35 ± 2.78%, while its 1–3 kDa peptide fraction was the most active (30.52 ± 0.01%) α-amylase inhibitor. Results based on the mean inhibitory responses showed that the LMW peptides were better α-amylase and α-glucosidase inhibitors compared to the unfractionated hydrolysates. The mode of inhibition was identified to be non-competitive for α-glucosidase, which indicate peptide binding to the non-active site of the enzyme. In contrast the peptides interacted with the active site of α-amylase because the mode of inhibition was competitive.

Conclusion: Findings from this study suggest that pea-protein derived peptides have the potential to be developed into functional foods and/or nutraceuticals for management of caloric intake with respect to T2DM. Future feeding studies using animal models and human volunteers are required to confirm the results obtained from this in vitro inhibition of enzyme activity.

4. **Optimization of Submerged Fungal Incubation Process for Production of Guar Protein Hydrolysate.** Jacob Zahler[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 world population expected to increase to 9 billion people by 2050, improvements in food production are of interest. The rapidly growing aquaculture industry is an efficient platform for production of animal protein with a feed to meat conversion ratio of 1:1, compared to 2:1 for poultry, 3:1 for swine, and 6-8:1 for cattle. Inhibiting the economic feasibility of aquaculture operations are the

volatile prices of aquafeed ingredients such as fish meal. Thus, interest has grown in utilizing plant-based protein sources for inclusion into aquadiets. Usage of plant-based protein sources are limited due to the presence of anti-nutritional factors, indigestible carbohydrates, lack of essential amino acids and low protein titers. Guar meal contains beneficial amounts of protein and residual galactomannan gum, however, also contains high levels of fibers and low amounts of amino acids which limit inclusion rates in aquadiets. Microbial fermentation has been shown to decrease levels of fibers in feeds while simultaneously concentrating protein and amino acids levels consequently increasing the nutritional value of feeds. Therefore, this research is aimed at reducing the amount of carbohydrates while increasing protein and amino acid titers using a submerged fungal process. We screened five fungal strains for their ability to degrade carbohydrate fractions while optimizing downstream processing procedures for increased protein concentration. The fungal incubation process was found to be effective, showcasing the potential applications of this technology on a variety of feedstocks. Overall, this process could be used for production of alternative plant-based protein ingredients for use in aquadiets.

5. **Effect of Physical and Biochemical Pre-treatment on Digestibility and Bioacessibility of Nutrients in Pulses.** Elisa Di Stefano[1], Chibuike C. Udenigwe[1], and Teresa Oliviero[2], [1]*University of Ottawa, Canada;* [2]*Wageningen University, The Netherlands*

Pulses play an important role in human diets worldwide, but their digestibility can be impaired by the presence of anti-nutritional factors such as phytic acid and phenolic compounds. Physical and biochemical pre-treatments have the potential to increase digestibility and bioaccessibility of pulse nutrients during digestion, besides positively affecting the sensory quality. In this study, the effects of germination, solid-state fermentation, heat treatment, mechanical disruption, and a combination of these treatments, were investigated in relation to pulse nutrients digestibility and bioaccessibility after *in vitro* gastrointestinal digestion. After pre-treatment, the five pulses selected for this study, faba bean, kidney bean, chickpeas, green lentils and yellow peas, were freeze-dried and ground into flour before analysis. Increase in germination time up to 5 days corresponded with increase in protein digestibility before simulate gastro-intestinal digestion, but didn't impact the bioaccessibility of proteins. Interestingly, physical pre-treatment of the pulses (grinding, soaking) and solid state fermentation significantly increased the digestibility of pulses compared to thermal treatment, but the thermal treatment provided significantly higher values for bioaccessibility of proteins. Overall, germination, and a combination of grinding and fermentation, were the most efficient pre-treatments in increasing digestibility of pulses before simulated gastro-intestinal digestion, while thermal pre-treatment provided the highest bioaccessibility of the same nutrients. This study highlights the importance of considering bioaccessibility as key factor when determining the nutritional value of pulses.

6. **A New Chromatographic Method for Simultaneous Quantification of Proteins and Phenolic Compounds from Oleaginous Meal.** Sara Albe Slabi[1], Christelle Mathé[2], Xavier

Framboisier[3], Arnaud Aymes[3], Olivier Galet[4], and Romain Kapel[3], *[1]Reaction and Process Engineering Laboratory UMR-7274, Avril Group, France; [2]Reaction and Process Engineering Laboratory, France; [3]Reaction and Process Engineering Laboratory UMR-7274, France; [4]Avril Group, France*

Currently, oleaginous meal is an attractive source of proteins for future human nutrition. Moreover, this by-product of oil extraction contains also a large amount of phenolic compounds like chlorogenic acid (sunflower meal) or sinapic acid (rapeseed meal). However, optimization of their co-extraction under aqueous conditions requires a suitable analytical tool. The commonly known methods for analysis of such biomolecules have drawbacks and none allows the quantification of both compounds simultaneously. Thus, the aim of this research was to develop a new chromatographic method to tackle this challenge. The first step was to establish optimal chromatographic parameters for elution of analytes. Subsequently, the method developed was tested for various operating conditions of extraction (pH, salt concentration) and two different oleaginous meals (sunflower, rapeseed). Finally, the results of protein quantification were compared with the reference Kjeldahl method and BCA test. As a result, a method developed from size exclusion chromatography (SEC) provided a satisfactory separation between proteins and phenolic compounds (different isomers of chlorogenic acid or sinapic acid). Furthermore, routinely used BCA assay in comparison to reference method results in high overestimation of protein concentration (BCA = (0.67±0.30) × Kjeldahl + (13.82±3.53), R^2 = 0.73), whereas, a new chromatographic method showed good correlation with Kjeldahl method (SEC = (0.99±0.09) × Kjeldahl, R^2 = 0.96). Thus, the SEC method offers a reliable and simultaneous quantification of proteins and phenolic compounds in liquid phase. This method would be successfully applied for monitoring of their extraction and purification process from sunflower and rapeseed meals.

7. **Understanding the Effects of Processing Conditions on the Extraction of Oil and Protein from Almond Flour.** Thaiza Serrano Pinheiro de Souza, Neiva Maria M. de Almeida, F.F.G. Dias, and Juliana M. Leite Nobrega de Moura Bell, *University of California-Davis, USA*

The enzyme-assisted aqueous extraction is an environmentally friendly process in which oil and protein can be simultaneously extracted from several oil bearing materials (i.e., soy, nuts, corn, and peanuts). The aim of this study was to investigate the effects of extraction conditions on oil and protein extraction from almond flour. In addition, the distribution of the extracted compounds among the fractions (cream, skim, and insoluble) was evaluated. A fractional factorial design (24-1 plus three central points) was used for a preliminary evaluation of the effects of pH (6.5–9.5), temperature (45–55°C), solids-to-liquid ratio (SLR) (1:12–1:8), and amount of enzyme (0.5–1.0%) on the extraction and separation of oil and protein from almond flour. Oil extraction yields from 61–75 % were observed within the range of parameters evaluated. At longer reaction times, increasing temperature from 45 to 55 °C increased the amount of free oil to 4.6–9.5 %. Lower oil yield in the skim (2.6–5.0 %), a desirable trait for protein functionality, was observed at higher SLR (1:8). In general, higher protein extraction yields (70–79 %) were observed at lower SLR (1:10–

1:12), with minimum increase at reaction times above 40 min. Those results indicate that higher oil and protein extraction yields can be achieved at lower temperature, reduced amount of water and enzyme, and shorter reaction times, within pH 6.5 to 9.5. Small increment in protein extractability was observed with the use of enzyme compared with the control (without enzyme). Understanding the effects of the enzyme use on the protein functionality will indicate further applications of the extracted protein.

8. **Peptide Mapping of Cryoprecipitated Proteins from Select Rosaceae Seeds.** Sahil Gupta, Valerie D. Zaffran, Tengfei Li, and Shridhar K. Sathe, *Florida State University, USA*

Rosaceae family contains several commercially important members including almond. Amandin is the major allergen in almond seeds. Sodium dodecyl sulfate polyacrylamide gel electrophoretic (SDS-PAGE) profiles of eleven from the investigated forty-seven Rosaceae seeds indicated differences. The objective of this study was to compare the peptide map of amandin-like protein in these eleven seeds with that of Nonpareil almond amandin. Cryoprecipitation method reported for almond amandin preparation was used to isolate the target protein from the selected seeds. In vitro digestion in 1.5 ml plastic micro-centrifuge tubes was done under final conditions: substrate protein (1 mg/ml), substrate to enzyme ratio 100:1 (w/w), buffer concentration 0.1 M, incubation temperature 37°C, and digestion time 10 minutes. For trypsin and chymotrypsin digestions, 0.1 M Tris-HC1 buffer (pH 8.1) and for pepsin digestions 0.1 M HCl were used. Enzyme action was stopped by adding an equal volume of SDS-PAGE sample buffer containing 2% (v/v) β-ME followed by 10 minute heating in a boiling water bath. The digested proteins were subjected to electrophoresis, Western and dot blots using anti-amandin murine monoclonal antibody (mAb) 4F10. Nonpareil amandin was hydrolyzed by pepsin, trypsin and chymotrypsin to yield 12-25 & < 10 KDa, 10-25 KDa and 10-34 KDa polypeptides, respectively. Seven of the tested proteins showed distinct peptide maps compared to Nonpareil amandin. Chymotrypsin, and not trypsin and pepsin, generated polypeptides were recognized by mAb 4F10 during blotting. Distinct peptide maps may indicate: distinct protein stretches, primary sequences, higher structures and/or variable enzymatic sites.

9. **Inhibitory Effects of Hydrolyzed Oat Proteins on Human LDL Oxidation their Bile Acids Binding Capacity.** Gabriela Campos and Apollinaire Tsopmo, *Carleton University, Canada*

Reduction in blood cholesterol is essential to the prevention and treatment of cardiovascular diseases. Many reports have demonstrated that peptides may influence bile acids that are major metabolites of cholesterol and facilitate their elimination in the feces through the formation of micelles that solubilize the cholesterol in the bile. The aim of this study was to evaluate the capacity of oat bran protein hydrolysates to bind bile acids and to prevent oxidative damage. Fifty grams of medium oat bran flour were treated with viscozyme (3 FBG/g) or cellulase (20 EGU/g) to hydrolyze polysaccharides. Proteins were solubilized at pH 10.0 followed by precipitation at their isoelectric point (pH 4.5). Extraction yields were 27% and 16% while, soluble protein contents were 70 and 60% for viscozyme and

cellulase treated flour isolates, respectively. Each protein sample was then hydrolyzed with five enzymes (protamex, alcalase, flavourzyme, pepsin, pepsin+pancreatin). In the bile binding assay, the alcalase hydrolysate of proteins from viscozyme-treated brans had the best activity and chelated taurodeoxycholic acid and taurocholate by 25.4 and 45.5 %, respectively. The same hydrolysate also possessed the highest peroxyl radical scavenging activity (495.5 ±13.4 µM TE/g). Anti-oxidative property was also determined based the ability of samples to prevent oxidation of LDL-cholesterol. Alcalase hydrolysate reduced the formation of hydroperoxides from 247.1 (control) to 47.6 µM H2O2/g. The most active hydrolysate was subsequently separated into eleven fractions by high performance liquid chromatography and fractions F5, F7, and F9 had the highest antioxidant activities as indicated by the peroxyl radical scavenging assay.

10. **Characterization of Soluble Proteins from Commercial Oat Millings.** Mallory E. Walters and Apollinaire Tsopmo, *Carleton University, Canada*

The aim of this study was to investigate the effect of processing on the extraction of extraction of proteins from oats, characterize the proteins and determine their functional properties. Six commercial fractions, including fine bran (FB), low bran (LB), medium bran (MB), whole flour (WF), 15% high glucan (15HG) and 20% high glucan (20HG), were used. Proteins were characterized using Fourier-transform infrared spectroscopy (FTIR), mass spectrometry (MS), scanning electron microscopy (SEM) and gel electrophoresis. MB had the highest (97.1 ± 3.9%) yield of extracted proteins, compared to 75.0–83.9% proteins observed for LB, FB or WF. Protein contents for 15HG and 20HG samples were about 4-fold lower which was due to their carbohydrate content (~75%). Mass spectrometry analyses showed that there were few differences in proteins composition of the samples. Avenin protein was not detected in FB and MB samples due to the relatively higher concentrations of 11S and 12S globulin. Fourier-transform infrared spectroscopy showed identical peaks for all samples, but additional peaks attributed to carbohydrates were present in the 15HG and 20HG samples. The gels formed by 15HG and 20HG proteins had smooth microstructures, while those from other samples had porous microstructures. The water holding capacity and solubility of high glucan samples were much greater for 20HG and FB samples. Overall, the results demonstrate that the milling processes have an effect on the structural and functional properties of oat proteins and that proteins extracted from fine bran have the most proteins and better functionalities.

11. **Physicochemical Properties of Rice Albumin with a Suppressive Function Against Hyperglycemia.** Aya Hamada[1], Shigenobu Ina[2], Nozomi Fujisawa[2], Ayaka Akima[3], Yusuke Yamaguchi[1], Makoto Akao[4], Hitoshi Kumagai[3], and Hitomi Kumagai[4], *[1]Nihon University, Japan; [2]College of Bioresource Sciences, Nihon University, Japan; [3]Kyoritsu Women's University, Japan; [4]Dept. of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, Japan*

Rice is a staple food in many countries and is one of the most widely consumed plant foods as a principal source of carbohydrates and proteins in Asian countries. We have already shown that rice albumin is indigestible and suppresses postprandial hyperglycemia probably by adsorbing glucose onto its unhydrolyzed large

molecule in the small intestine. Although rice albumin possesses useful physiological properties like hypoglycemic effect, it is important to have desirable physicochemical properties such as foaming, emulsifying and thermal properties in order to process it as functional foods. The present study aimed to evaluate the physicochemical properties of rice albumin. Foaming property was measured by the bubbling method, and emulsifying property was by the turbidity method. Thermal properties were evaluated by measuring the α-amylase inhibitory activity at various temperatures and the endothermic peak observed by differential scanning calorimetry during heating. The temperature of endothermic peak is considered to be the denaturation temperature. The foaming property of rice albumin was similar to that of dried egg white, and the emulsifying property was kept high in the range of pH 3-8. The α-amylase inhibitory activity of rice albumin was maintained almost 100% even when it was heated at 121°C for 20 minutes at a concentration of 0.1% (w/w). The temperature of endothermic peak of rice albumin was higher than that of bovine serum albumin and whey protein. These results suggest that rice albumin has excellent functionality, with high heat resistance, and can be a food material suitable for manufacturing functional food.

12. **Deamidation of Water-soluble Wheat Gliadin by Cation-exchange Resins.** Hanae Nakamura[1], Sumika Ochiai[1], Ryusuke Abe[1], Yusuke Yamaguchi, Makoto Akao[2], Hitoshi Kumagai[3], Reiko Urade[4], and Hitomi Kumagai[2], *[1]Nihon University, Japan; [2]Dept. of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, Japan; [3]Faculty of Home Economics, Kyoritsu Women's University; [4]Kyoto University, Japan*

Deamidation is often effective to improve protein functions such as water solubility and emulsifiability. We have developed a method to deamidate food proteins without causing hydrolysis of peptide bonds using carboxylate-type cation- exchange resins. Deamidation of gliadin by this method exhibited improvement of water solubility, digestibility and foaming property as well as reduction in allergenicity. However, despite the use of gliadin extracted with 60% ethanol, the solubility of gliadin in this solvent was low, and the degree of deamidation in 60% ethanol was only 28%. In recent years, a novel method to prepare water-soluble gliadin was developed by using wheat-flour dough containing NaCl. Gliadin extracted by this method dissolved in water at a high concentration. Since gliadin in water is considered to have different conformation from that in 60% ethanol, treatment of gliadin in water with cation resins may enhance the efficiency of deamidation. In this study, we prepared water-soluble gliadin from wheat-flour dough containing NaCl and attempted to deamidate the gliadin in water with cation-exchange resins. The reaction condition was optimized by changing the amount of cation-exchange resins of the carboxylate type added to gliadin solution. The degree of deamidation was improved with increasing the amount of resins and reached about 50% when the amount of resins was 1.0 g/mL in gliadin solution.

13. **Protease Hydrolysis to Alter the Functional Properties of Proteins.** Kelly Gregory, Caroline H. Best, Deborah Winetzky, and Chris Penet, *Bio-Cat, USA*

In food processing, proteins can be hydrolyzed by protease enzymes to alter their functional properties, such as solubility, viscosity, water holding capacity, etc. Soy protein isolate, pea, hemp, brown rice and whey proteins were hydrolyzed using a blend of exo- and endo-acting proteases and their functional properties and effect on flavor evaluated. Each protein was treated with a blend of exo- and endo-proteases from Aspergillus oryzae for two hours at 50°C. The hydrolyzed proteins were spray dried then evaluated for degree of hydrolysis, flavor, solubility, viscosity, water holding capacity, and foaming. A free amino nitrogen assay using O-phthaldialdehyde was used to measure degree of hydrolysis. Flavor was evaluated by a taste panel. Solubility was determined by the amount of protein in solution as a percentage of total protein. Water holding capacity was determined as the mass remaining after soaking in DI water and centrifugation. Foaming was measured as the foam height over time after agitation, and viscosity was measured using a Brookfield viscometer. The degree of hydrolysis varied with protein type. 62% of taste panelists preferred the flavor of hydrolyzed proteins over unhydrolyzed proteins. Hydrolysis with the protease blend increased the solubility of all proteins except whey protein, and decreased foaming, water holding capacity and viscosity. The protease blend altered the functional properties of the proteins tested without producing off flavors.

14. **Substitution of Naturally Occurring Bromelain using a Blend of Proteases.** Caroline H. Best, Kelly Gregory, and Chris Penet, [1]*Bio-Cat, USA*

Bromelain is a naturally occurring enzyme extracted from pineapples that contains mixture of proteolytic enzymes that function when a cysteine side chain is present. Bromelain is commonly used in various food processing applications such as baking and meat tenderization, the processing of animal feed, and textiles. The availability of bromelain fluctuates due to the availability of pineapples making it prudent to find a substitute for bromelain. The performance of bromelain in these applications can be mimicked using a blend of proteases with a wide pH and temperature range. Hydrolysates of soy, corn, and whey were analyzed using several methods including degree of hydrolysis (OPA method), Primary Amino Nitrogen (PAN) and SDS-PAGE to compare the performance of the protease blend to that of bromelain. The resulting analysis determined that the blend of proteases, when introduced to a high protein substrate which would be similar to that used in the food processing and animal feed, showed similar properties to bromelain hydrolyzed protein and often exceeded the performance of bromelain. It needs to be duly noted that the blend of proteases, while it does mimic the performance of bromelain has not been tested nor proven to have the same anti-inflammatory properties that are often associated with bromelain and therefore cannot be used in a dietary supplement or in medical grade applications.

15. **Orally Administered Ovotransferrin Preserves Bone Microarchitecture in Ovariectomized Rats.** Nan Shang and Jianping Wu, *University of Alberta, Canada*

Egg white ovotransferrin is a member of transferrin family. Our previous study reported for the first time its ability to stimulate osteoblast (bone forming cells) activity while to inhibit osteoclast (bone resorption cells) activity. The overall objective of this study is to investigate its in vivo efficacy using ovariectomized rat, a widely used animal model to study osteoporosis. Three-month-old female ovariectomized Sprague-Dawley rats were orally administered with ovotransferrin for 3 month. The bone microarchitecture, bone volume, trabecular number, thickness, and trabecular separation was tested by micro-CT scan and analyzed by vendor-supplied software. Serum bone alkaline phosphatase, serum osteocalcin, and serum parathyroid hormone was tested by Elisa assay. Our results showed that ovotransferrin increased the parameters of bone microarchitecture in ovariectomized rats, which suggested the positive effects of ovotransferrin in preventing osteoporosis. In addition, serum bone alkaline phosphatase, osteocalcin, and parathyroid hormone were increased in ovotransferrin treatment indicating the potential of ovotransferrin on promoting bone growth. In conclusion, this study showed that oral administration of ovotransferrin could not only inhibit osteoporotic bone loss, but also promote bone growth at the same time, suggesting ovotransferrin could be used as a nutraceutical for osteoporosis prevention.

16. **Converting Corn Distillers Grain Proteins to High-value Antioxidants.** Ruijia Hu[1], Wei Wu[1], and Yonghui Li*[2], [1]*Kansas State University, Grain Science and Industry, USA;* [2]*Kansas State University, USA*

About 40% of U.S. corn is used for ethanol production, resulting in 90 billion pounds of distiller's grains (e.g., DDGS) and corn gluten meals (CGM) each year. Novel and more suitable value-added uses of these high protein byproducts are key to the economic viability of fuel ethanol production. Antioxidants are commonly added to human foods and beverages, pet foods, animal feed, as well as many industrial products. A rising trend in food and feed manufactures and consumers is the gradual replacement of synthetic antioxidants with natural antioxidants. Corn proteins 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. Enzymatic hydrolysis could be a feasible approach to produce corn peptides with targeted functional properties. The objectives of this study were to: 1) study the effect of various variables (types of proteases, enzyme to protein ratio, reaction time) on the antioxidant activity of DDGS and CGM protein hydrolysate; 2) understand the composition-structure-activity relationships of the hydrolysates; and 3) produce high-performance antioxidants from DDGS and CGM. We found that both DDGS and CGM were feasible sources for antioxidant production with antioxidant yield of above 60% and 73%,

respectively. Alcalase and Bromelain were the most effective enzymes with respect to antioxidant yield and activity among the ten proteases evaluated. Membrane ultrafiltration, size exclusion chromatography, and ion exchange chromatography were further used to fractionate the hydrolysates to understand their composition-activity relationships.

17. **Single-shot Top-down Proteomics with Capillary Electrophoresis-electrospray Ionization-tandem Mass-spectrometry for Identification of 570 Escherichia Coli Proteoforms.** Rachele A. Lubeckyj, *Michigan State University, USA*

Capillary zone electrophoresis-electrospray ionization-tandem mass spectrometry (CZE-ESI-MS/MS) has been recognized as an invaluable platform for top-down proteomics. The scale of top-down proteomics using CZE-MS/MS is still limited due to the low loading capacity and narrow separation window of CZE. In this work, for the first time we systematically evaluated the dynamic pH junction method for focusing of intact proteins during CZE-MS. The optimized dynamic pH junction based CZE-MS/MS approached 1-µL loading capacity, 90-min separation window and high peak capacity (~280) for characterization of an Escherichia coli proteome. Single-shot CZE-MS/MS identified about 2,800 proteoform-spectrum matches, nearly 600 proteoforms, and 200 proteins from the Escherichia coli proteome with spectrum-level false discovery rate less than 1%. The number of identified proteoforms in this work is over three times higher than that in previous single-shot CZE-MS/MS studies. Truncations, N-terminal methionine excision, signal peptide removal and some post-translational modifications including oxidation and acetylation were detected. Methods An automated CZE-MS system was used, including a LPA coated capillary (50-µm i.d./360-µm o.d., 1 meter long), a CE autosampler, a commercialized electro-kinetically pumped sheath flow CE−MS interface (CMP Scientific) and a QE-HF mass spectrometer (Thermo Fisher Scientific). Sample injection was performed via pressure. The background electrolyte for all samples was 5% (v/v) acetic acid and the sheath buffer was 0.2% (v/v) formic acid containing 10% (v/v) methanol. 30 kV for Vseparation and 2 kV for electrospray ionization. Impact Results demonstrated the largest loading capacity, highest peak capacity, and identification of single-shot CZE for top-down characterization of complex proteomes.

18. **Protein Digestibility and Quality Determined using Two *in vitro* Methods in Cooked, Baked and Extruded Pulses.** Adam J. Franczyk, Gerardo Medina, Matthew G. Nosworthy, Jason Neufeld, and James D. House, *University of Manitoba, Canada*

Protein quality evaluations currently require that an animal bioassay be performed to determine true protein digestibility (TPD). This measure is used to calculate the protein digestibility corrected amino acid score (PDCAAS) prior to qualifying for a protein content claim. Several *in vitro* assays evaluating protein digestibility that currently reside in literature have not been used to assess protein quality, hence this study was undertaken to evaluate the pH-drop method and two-step digestion method to determine PDCAAS *in vitro* on ten pulse classes. Pulses included a variety of beans (faba, black, navy and red kidney), peas (yellow and green), lentils (red and green) and chickpeas that

were cooked, baked or extruded to emulate consumer usage. The *in vivo* component utilized Sprague Dawley rats (n=320, ~70g) fed a diet consisting of 10% crude pulse protein (or casein as a control) to evaluate TPD and PDCAAS. Previous correlations between the pH-drop method and *in vivo* rat digestibility was used to calculate *in vitro* protein digestibility (IVPD-1), whereas the two-step digestion (IVPD-2) was determined by measuring the remaining nitrogen from digestion and measured through an o-phthalaldehyde (OPA) spectrophotometric assay. The IVPD-1 (R^2=0.5987) slightly outperformed IVPD-2 (R^2=0.5329) when both are correlated to TPD; however, when subjected to the same amino acid score (AAS) used to calculate PDCAAS, both the pH-drop (R^2=0.8415) and the two-step (R^2=0.8397) revealed a strong correlation to PDCAAS. This relationship suggests that either method to evaluate protein digestibility may be reliable to calculate PDCAAS *in vitro*, thus reducing the time, expense and ethical quandaries that currently apply to evaluating protein quality content claims.