



106th AOCS Annual Meeting and Industry Showcases

Protein and Co-Products Division Technical Program Abstracts

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The presenter is the first author or otherwise indicated with an asterisk ().*

PCP 1: Analysis and Characterization of Protein and Protein Hydrolysates for Utilization: Relevance and Caveats of the Methods

This session is sponsored in part by DuPont Nutrition & Health

Chairs: P. Kerr, Solae LLC, USA; C.C. Udenigwe, Dalhousie University, Canada; and E. Krul, Solae LLC, USA

Mass Spectrometry of Proteome and Biomarkers for Food and Agricultural Applications. S. Chen¹, J. Parker¹, K. Balmant¹, M. Zhu¹, T. Zhang¹, T. Ma¹, M.J. Yoo¹, J. Sheffield², and N. Taylor², ¹University of Florida, USA, ²Danforth Center, USA.

Rapid advances have been made in the field of mass spectrometry (MS) over the past decades. High sensitivity, high resolution and high throughput MS technologies have become the workhorse for both global and targeted proteomics. The overall objective of our research is to develop and apply the modern technologies to enhance food quality and agricultural productivity. Using high-resolution 2-D gel electrophoresis, we resolved proteins extracted from fibrous and tuberous roots of cassava plants. Gel image analysis revealed an average of 1467 protein spots on the fibrous gels and 1595 spots on the tuberous gels. Differentially expressed proteins were sequenced using quadrupole time-of-flight MS. Proteomics of the cassava roots is an important step towards understanding of the mechanisms underlying root development and enhancing root protein content and quality. In another project, isotope labeling based multiple dimensional liquid chromatography MS was used to identify and characterize protein molecular switches in stomatal guard cells. Several posttranslational molecular switches were determined to play essential roles in carbon fixation and defense, thus directly related to yield and bioenergy. In conclusion, modern MS technologies are essential for analysis and characterization of protein and peptides for a variety of utilization.

Using Synchrotron-based Infrared Microspectroscopy for Plant-based Feed and Food Protein and Nutrition Study. P. Yu, University of Saskatchewan, Canada.

Traditional wet analytical methods often result in destruction or alteration of food and feed intrinsic structures during processing for analysis. Synchrotron radiation-based infrared microspectroscopy has been developed as a non-destructive analytical technique. This technique, taking advantages of synchrotron light brightness (million time brighter than sun), is capable of exploring chemistry, composition, environment and

structure of a biological tissue without destruction inherent structures at high spatial resolutions. The objective of this article is to show the potential of the synchrotron-based technique, which can be used to study food or feed protein structure in relation to utilization and availability. Several applications are reported on using the synchrotron-based techniques in combination with other methods to study the effects of gene-transformation, heat related processing (eg. autoclaving, pressure toasting), bio-ethanol and bio-oil processing on plant-based food and feed protein structure changes. Synchrotron-based analytical technique makes contributions to advances in protein science and feed and food science technology.

Transport of Food Derived Peptides Important in Heart and Mental Health Across the Blood Brain Barrier: A New Model. M. Hayes¹ and T.E. Lea², ¹Teagasc Food Research Centre, Ireland, ²University of Life Sciences NMBU, Norway.

The aim of the project is to determine the bioavailability of prolyl endopeptidase and acetylcholinesterase inhibitory peptides generated from seaweed and boarfish. Alzheimer's disease is the most common cause of dementia in our aging society. PEP and AChE inhibitory peptides are thought to assist in the prevention of amyloid plaque formation in the brain which is linked to the development of Alzheimer's.

The blood brain barrier (BBB) remains a significant hurdle for treatment of central nervous system and brain diseases. PEP and AChE inhibitory peptides generated previously using in silico analysis and simulated digestions were chemically synthesised and labelled with fluorescent tags. The bioavailability of peptides was determined using an in vitro model. A reliable in vitro model system of transcytosis was established and tested to assess the potential of food derived peptides to cross the BBB. Transport across the BBB was determined using a number of methods (microscopy). UPLC-MS was used to determine whether apical to basolateral transcytosis of peptides occurs.

Digestibility and Allergenicity of Processed Legume Proteins by a Dynamic *in vitro* Digestion System.

L. L'Hocine, M. Pitre, Y. Arcand, I. Mainville, and A. Achouri, Agriculture and Agri-Food Canada, Canada.

The persistence of isolated proteins in simulated gastric fluid is now widely recognized as not a reliable predictor of their allergenic potential, because they are tested out of context, not considering the effects of processing, and matrix interactions. The evaluation of protein digestibility within their matrices provides more correlative result and is yet to be assessed. The objective of this study was to examine the effect of roasting and boiling, and fat content and structure of the matrix on digestibility and subsequent allergenicity of peanut and bean proteins, using a physiologically relevant and fully computerized dynamic *in vitro* human digestion model. The patterns of allergens release and breakdown were followed by electrophoresis and IgE-based immunoassays. Results revealed important differences in allergens digestion profiles between boiled and roasted legume proteins. The latter demonstrating an increased resistance to digestion. Increasing the fat content and emulsification of legume matrices delayed considerably the digestion of proteins causing the persistence of IgE-binding peptides in the duodenal digest. This study provides the basis for the development of integrative methods for

assessing the allergenic risks posed by conventional and novel food processing technologies.

Storage Stability of Food Protein Hydrolysates.

Q. Rao¹, T.P. Labuza², and J. Zhao¹, ¹Florida State University, USA, ²University of Minnesota, USA.

In recent years, mainly due to the specific health benefits associated with 1) the released bioactive peptides, 2) the potential reduction of protein allergenicity, and 3) the improved protein digestibility and absorption, the utilization of protein hydrolysates in functional foods and beverages has significantly increased. Although the specific health benefits from different hydrolysates are somewhat proven, the stability of these benefits is debatable during distribution, storage and consumption. In this presentation, we will discuss 1) the quality changes in different food protein hydrolysates during storage; 2) the resulting changes in the structure and texture of three food matrices, i.e., low moisture foods (LMF, $a_w < 0.6$), intermediate moisture foods (IMF, $0.6 = a_w < 0.85$), and high moisture foods (HMF, $a_w = 0.85$); and 3) the potential solutions to improve storage stability of food protein hydrolysates. Additionally, it must be noted that there is a great need for evaluation of biofunction availability of bioactive peptides in food protein hydrolysates during storage.

PCP 2: Biocatalysts in Processing of Proteins and Co-Products

This session is sponsored in part by DuPont Nutrition & Health

Chairs: B.P. Lamsal, Iowa State University, USA; N. Shah, DuPont Health and Nutrition, USA; and R. Green, POS BioSciences, Canada

Enzymatic Protein Hydrolysates: From Improved Food Protein Functionality to Bioactive Peptides.

R.E. Aluko, University of Manitoba, Canada.

This presentation will examine the use of proteolytic enzymes as processing tools to develop protein and peptide products with superior emulsifying and antihypertensive properties, respectively. To prepare better emulsifying proteins, canola proteins were treated with Proleather FG-F for 2.5 and 10min. Using a mayonnaise formulation that contained 70% (w/w) oil, the hydrolyzed canola proteins substituted up to 50% of the egg yolk weight as an emulsifier in comparison to only 15% for the unhydrolyzed protein. However, the presence of canola proteins reduced whiteness and viscosity of the mayonnaise. For antihypertensive peptides, hemp seed proteins were subjected to pepsin and pancreatin digestions to produce a protein hydrolysate (HPH) that inhibited in vitro activities of renin-angiotensin system enzymes. During long-term (8 weeks) oral administration to spontaneously hypertensive rats (SHR), blood pressure increases were attenuated better by the HPH when compared to the unhydrolyzed hemp seed protein. Analysis of the SHR plasma showed significant reductions in the activities of renin and angiotensin converting enzyme, the principal enzymes that are responsible for human systemic hypertension. Thus enzymatic protein hydrolysis offers a useful tool for the fabrication of novel protein and peptide ingredients.

Fractionation of Rapeseed from Oil Extraction to Minor Products. S. Hruschka, GEA Westfalia Separator Group GmbH, Germany.

Starting with the composition of the seed, the presentation illustrates the breakdown of the seeds, mainly by mechanical processes.

Since the demand for digestible vegetable proteins is increasing and different factors have a negative impact on the oil price, too, other by-products behind the oil and new processes are becoming more interesting for oil producers and oil refining costs are more and more in focus.

Different steps will be illustrated: 1) Seed and pre-treatment, 2) De-oiling, 3) Oil and derivative processing, 4) Cake and flakes processing and finally

the By-products from both sides: 5) those from the oil fraction and those from the cake or expeller side. The oil and the protein content, the hull thickness and therefore the fibre content, the sinapin and phytic acid content, the glycosinolate content etc. are not only given and fixed in the incoming raw material. All this can be influenced already with the first step: the pre-treatment and the kind of oil extraction.

Consequently, the oil refining starts between 10 ppm phosphorous or < 1000ppm phosphorus in the oil depending on the extraction technique, but what is not in the extracted oil remains in the cake or expeller, meaning phosphorus, too. That influences the percentage of the substances and behaviour of each fraction and ultimately the processes itself.

Proteins and Bioactive Peptides Produced from Hard to Cook Common Bean Improved Markers Related to Diabetes. E. De Mejia¹, M. Oseguera^{1,2}, and S. Amaya², ¹University of Illinois, USA, ²University of Queretaro, Mexico.

Common bean (*Phaseolus vulgaris* L.), a legume with high protein concentration, constitutes a basic source of nutrition in several countries around the world. The hard-to-cook (HTC) phenomenon is characterized by long cooking times and reduced sensory properties affecting its commercialization. The objective was to study the effect of alcalase and bromelain hydrolysates, and their bioactive peptides, produced from dehulled hard-to-cook beans on markers related to diabetes *in vitro*. Bean protein concentrates were hydrolyzed with either alcalase or bromelain and fractionated into five peptide fractions using an ultrafiltration membrane system. Potential anti-diabetic effects were evaluated by α -amylase, α -glucosidase and dipeptidyl peptidase-IV (DPP-IV) inhibition. Peptides identified were LLSL or LSLL, QQEG, NEGEAH and INAKNI. Fraction < 1 kDa Pinto Durango-bromelain showed the best inhibition of α -amylase ($49.9 \pm 1.4\%$) and for α -glucosidase Pinto Durango-alcalase (76.4 ± 0.5). Hydrolysates from HTC beans inhibited enzymes related to diabetes management and the smallest peptides were the most potent. It is concluded that bioactive peptides produced and identified in HTC beans offer a potential for incorporation into

functional and nutraceutical foods.

Effects of Co-Products of Enzyme-assisted Aqueous Extraction of Soybeans on Ethanol Production in Corn Fermentation. J.K. Sekhon¹, K. Rosentrater¹, T. Wang¹, L. Johnson¹, and S. Jung^{1,2}, ¹Iowa State University, USA, ²California Polytechnic State University, USA.

Biofuels can reduce dependency on non-renewable sources of fuels and offer multiple environmental benefits. However, more efficient methods of biofuel production are needed to effectively compete with petroleum fuels in the market place. One such approach is an integrated corn-soy biorefinery, which combines components from soybean processing into corn-based ethanol processing. Enzyme-assisted aqueous extraction processing (EAEP) is an environmentally-friendly alternative to chemical and mechanical extraction methods which facilitate ~97% oil recovery from soybeans. The present study utilized soy skim (protein rich) and insoluble fiber (IF; carbohydrate rich), co-products of EAEP, in dry-grind corn fermentation, and investigated the effect of adding 1) soy skim and IF separately or together, and 2) pretreated or untreated IF on ethanol production. Maximum ethanol production was achieved when corn, untreated IF and skim were slurried together (corn-to-untreated IF ratio 1:0.2; skim-to-untreated IF ratio 4:1, on dry basis) and fiber hydrolyzing enzymes were added. This resulted in 20% and 3% increase in ethanol yield and ethanol production rate, respectively, and a 44 h decrease in fermentation time compared to corn-only fermentation.

Technoeconomic Analysis of Bioethanol and Co-Product Production from Triticale Feedstocks in Western Canada. E.K. Mupondwa¹, X. Li¹, J.P.D. Wanasundara¹, and L. Tabil², ¹Agriculture and Agri-Food Canada, Canada, ²University of Saskatchewan, Canada.

Canada's cellulosic biorefinery concept is predicated on the country's increased commitment

to the production of biofuels and co-products from low-cost cellulosic feedstocks that do not require agricultural intensification and direct competition with sources such as grains used for food and livestock feed. Dedicated crops such as triticale offer both grain and cellulosic biomass that are expected to have a huge impact on the development of Canada's biorefinery concept and the generation of new bio-products. This study provides a technoeconomic analysis of bioethanol and co-product production from triticale feedstocks in western Canada. A comprehensive technoeconomic evaluation of the optimization process for the production of co-products is provided, including an empirical characterization of the complexity of conversion economics from the production of bioethanol and coproducts from triticale grain starch (requiring enzymes to hydrolyze starch to glucose before fermentation) versus lignocellulosic substrates (requiring more extensive processing to release polymeric sugars in cellulose and hemicellulose).

A New Enzyme Cocktail for Degradation of Dietary Fibers in Palm Kernel Cake. T.T. Hansen, D. Pettersson, M. Plambech-Nielsen, and U. Madsen, Novozymes, Denmark.

Palm kernel cake is a by-product from the production of palm kernel oil. The cake is used as an inexpensive fiber feed, best suited to ruminants, since the protein content is relative low.

A new enzyme cocktail consisting of different mono-component carbohydrases have shown to be effective in hydrolyzing the majority of the fibers to fermentable sugars. These can be converted to valuable ethanol and the remaining cake will have an increased content of protein, and a low amount of fibers. This will allow the cake to be used as a value added product in feed for monogastric animals.

PCP 3/SCC: Strategies in Advanced Utilization of Proteins and Peptides

This session is sponsored in part by DuPont Nutrition & Health

Chairs: H.R. Ibrahim, Kagoshima University, Japan; H. Kumagai, Nihon University, Japan; Z. He, USDA, ARS, SRRC, USA; and P. Romanowski, Society of Cosmetic Chemists/Element 44 Inc., USA

Development of a Protein-rich, Novel Fermented Milk/Cereal Product as a Delivery Vehicle for Micronutrients and Bioactive Compounds. N.M. O'Brien and T.P. O'Connor, University College Cork, Ireland.

In 2014 it is estimated that 805 million people were food insecure, the vast majority in developing countries. Many of these individuals receive humanitarian assistance from organisations such as World Food Programme and other NGOs. These agencies commonly distribute Special Nutritional Products including Fortified Blended Foods (FBFs) targeted at young children to alleviate malnutrition. Currently used FBFs are primarily composed of dried legume/cereal blends which act as a delivery vehicle for fortified micronutrients. These FBFs are then reconstituted by heating with water and consumed as porridge. We are developing a novel FBF using dried fermented buttermilk/cereal as a delivery vehicle for micronutrients. Our product is rich in high quality dairy protein and is an excellent and palatable delivery vehicle for fortified micronutrients. Additionally, the product is also ideally suited as a delivery vehicle for other classes of bioactive compounds, including bioactive peptides, targeted at selected groups, e.g., elderly individuals. Bioactive peptides examined in this project will be chosen based on their favourable biological effects, resistance against gastrointestinal digestion and passage through enterocytes and caco-2 cell monolayers.

Proteomic Analysis of Eggshell Membranes. M.T. Hincke and T. Ahmed, University of Ottawa, Canada.

Avian eggshell membranes (ESM) play an essential role in the formation and structure of the eggshell and act as a barrier that protects the egg against bacterial invasion and spoilage. It is essential to characterize the ESM proteome in order to understand the mechanisms of ESM function. In our study we evaluated different extraction strategies including conditions of acid, base, salt and reducing agents, in order to solubilize the membrane fibers to identify its protein constituents using RP-nanoLC and ES-MS/MS proteomics. In total, we identified 180 proteins in the different extracts and solubilizates. We identified proteins involved in DNA packaging

and protein - DNA complex assembly including H1, H2A, H2B, H3, and H4. In addition, various members of collagen superfamily have been identified including a1 chain of collagen VIII and X, a2 chain of collagen V, and a6 chain of collagen IV. Proteins that are involved in cell adhesion have been also identified including cadherin 1, fibronectin, tenascin C, and neurotrimin. Further, antimicrobial proteins for instance gallinacin -9, -10, -11, gallin 1, β 2 microglobulin, and histone H2B are present. The results of this study will guide further efforts to exploit the bioactive elements of the ESM for therapeutic applications.

Impact of Dietary β -conglycinin on Adiposity and Insulin Sensitivity in Obese and Type 2 Diabetic Rodents. K. Koba¹, K. Kawabeta¹, T. Noda¹, N. Tateiwa¹, S. Tamaru¹, and M. Sugano², ¹University of Nagasaki, Japan, ²Kyushu University, Japan.

We previously demonstrated that β -conglycinin (β -CON), a component of soy protein (SOY), not only exerted lipid-lowering effect but increased insulin sensitivity in SD rats. Therefore, we examined the effect of β -CON in obese and diabetic animals. We prepared the diets containing either 20% casein (CAS), or CAS replaced 50% with SOY or β -CON. First, obese OLETF rats (6 wk-old) were fed with the diets for 13 weeks, and insulin tolerance test (ITT, 0.75IU/kg) was performed in week 12. The result suggested that SOY and more so β -CON increased insulin sensitivity, compared with CAS in obese rats. Also, β -CON, compared with CAS decreased visceral adipose tissue weight and liver triglyceride level, associated with an increase of serum adiponectin level. Then, we examined the β -CON effect using type 2 diabetic KK-Ay mice in two feeding trials (33 days in Exp. 1 & 14 days in Exp. 2). ITT (1IU/kg) was performed on day 28 and day 7, respectively and found that β -CON tended to modulate insulin sensitivity only in Exp. 1. The lipid-lowering effect of β -CON was observed in both experiments, but the effect was clearer in Exp. 2. Although the β -CON effects were varied depending on the experimental condition, the outcome of the present study suggested the beneficial effects of β -CON that prevent metabolic syndromes.

Occurrences of Food-derived and Endogenous Pro-hyp in Blood and Tissue and Their Function to Fibroblast. K. Sato, Kyoto University, Japan.

Human trials have demonstrated that ingestion of collagen hydrolysate improves skin and joint conditions. Occurrences of food-derived collagen peptides, Pro-Hyp in human blood after ingestion of collagen hydrolysate have been also reported. The Pro-Hyp enhance growth of primary cultured mouse skin fibroblast on collagen gel. The objectives of the present studies were to examine occurrences of these peptides in damaged tissues and elucidate how Pro-Hyp triggers growth of the fibroblast. Skin disc was removed from mice. Pro-Hyp content in the damaged and normal skin and ear were determined by LC-MS/MS. Fibroblasts, which were migrated from skin disc (primary cultured) and subcultured, were incubated in the medium containing Pro-Hyp.

Pro-Hyp content significantly increased in the damaged skin and ear compared to normal ones. The primary cultured fibroblast incorporated Pro-Hyp in dose dependent manner. On the other hand, subcultured fibroblast did not. Expression of peptide transporters (PEPT1, 2, Ci1) decreased after subculture. In addition, tissue stem cell marker, p75NTR was expressed in the primary cultured cell, but it rapidly decreased after the subculture. Thus, endogenous and also food-derived Pro-Hyp might specifically enhance growth of the fibroblast for wound healing process.

Intestinal Absorption of Bioactive Peptides: Detection and Visualization. T. Matsui, Kyushu University, Japan.

Studies on bioactive peptides regarding the prevention of lifestyle-related diseases continue to be one of the growing fields for alternative medicinal food sciences. It has been proven that the intake of small peptides or protein hydrolysates improved promoting high blood pressures in mild-hypertensive subjects. Our recent studies demonstrated additional health-beneficial effects such as anti-atherosclerosis [1] and vasorelaxation by the suppression of Ca²⁺-constriction cascades [2,3]. However, arguments on the absorption of small peptides such as di- and tri-peptides via PEPT1 transport have been raised, because of their lack of absorption in pharmacokinetic studies. To overcome these issues, stable-isotope labeling and chemical derivatization techniques combined with LC-tandem MS have been proposed in Caco-2 cell and/or animal experiments, permitting highly sensitive detection at

>50fmol/mL-plasma [4,5]. In addition, MALDI-imaging technique [6] allowed a visualization of transporting process of small peptides across rat intestinal membrane by their characteristic *m/z*, providing information on their distinct proteolytic degradation during absorption.

[1] *Br. J. Nutr.*, 103, 309 (2010).

[2] *Biochim. Biophys. Acta*, 1840, 3073 (2014).

[3] *PLoS ONE*, 9, e88975 (2014).

[4] *Anal. Biochem.*, 414, 109 (2011).

[5] *Anal. Chem.*, 85, 4289 (2013).

[6] *Anal. Chem.*, 85, 10033 (2013).

Appetite Suppressive Peptides Derived from Soybean Involvement of Enteroendocrine System.

H. Hara, S. Nakajima, and T. Hira, Hokkaido University, Japan.

Soybean seed is a good source of dietary protein as well as oil source. Recently, we found that a peptide in soybean b-conglycinin suppresses food intake in rats and hunger in healthy human with increasing secretion of cholecystokinin (CCK), which is a gut hormone produced from the enteroendocrine I-cells. We tried to identify the responsible peptides for stimulation of CCK release in soybean protein, and have got a tridecapeptide in b-conglycinin b subunit (b51-63). The amino acid sequence of this active peptide is VRIRLLQRFNKRS, that includes four arginine residues. Also, we have identified a receptor for b51-63, which is calcium-sensing receptor (CaSR), a GPCR, expressed on the enteroendocrine cell line STC-1. The soybean peptide strongly stimulates CCK release in rats after an injection into the duodenum, and suppresses food intake after overnight fast. The suppression of food intake completely depends on CCK action, which confirmed by a CCKA-receptor blocker. By a double blind crossover study in healthy individuals, a protease hydrolysate of soybean b-conglycinin suppresses hunger sensation and induces transient satiety in post-absorptive state compared with a whole soybean peptide preparation. The soybean b-conglycinin peptide may apply treatment of obesity and diabetes patients.

Bioactive Peptides from Goat Milk with New Promises for Skin Health. H.R. Ibrahim, Kagoshima University, Japan.

Today, bioactive peptides are at the top of the skincare ingredients list. Bioactive peptides are generated by enzymatic processing of precursor proteins. Their potent biological effects have attracted attention for applications in skin health.

Bioactive peptides released from milk proteins have gained increasing attention. These peptides exert immunomodulatory, antibacterial and antioxidant activities. In contrast to bovine milk, goat milk is gaining intensifying importance due to health problem associated with bovine milk allergy. In this study, we explore novel bioactive peptides generated by gastrointestinal digestion simulation of goat milk proteins. Several peptides exhibited potent superoxide anion scavenging activity as well as remarkable ability to reduce DPPH radicals. Certain peptides showed strong antimicrobial activity against skin pathogens, *P. acnes* and *C. minutissimum*. MALDI-TOF-MS allowed the identification of active peptides derived mostly from various caseins as well as lactoferrin and β -lactoglobulin from whey. This finding is the first to describe that goat milk possesses multiple

biopeptides, act as potent antioxidant protection and will help fight skin infection, that offer a fascinating opportunity for their potential skincare applications.

Protein and Peptide Use and Effectiveness in Topical Cosmetic Applications. P. Romanowski, Element 44 Inc., USA.

A variety of proteins and peptides have been used in cosmetic formulations for years for different effects. While these ingredients have widespread consumer appeal and often have laboratory data supporting their use, the practical effectiveness is more difficult to prove. In this talk we will focus on some of the most popular proteins and peptides used in anti-aging cosmetics including neurotransmitter inhibitors, signal peptides, carrier peptides, and enzyme inhibitors.

PCP 4: New Processes, Emerging Sources, Alternative Proteins

This session is sponsored in part by DuPont Nutrition & Health

Chairs: K. Liu, USDA, ARS, USA; H. Wang, Iowa State University, USA; and L. Jiang, Northeast Agricultural University, China

Canola Protein Nanoparticles: Preparation by Cold Gelation and Application in Delivery of Bioactive Compounds. J. Wu and A. Akbari, University of Alberta, Canada.

Encapsulation of bioactive compounds is an emerging technique to protect them in harsh condition of food processing or in the body. Increasing absorption and their bioavailability are other objectives of delivery systems. In this study, canola proteins were used to prepare nanoparticles through the cold gelation method. The results showed that lower CaCl₂ concentrations and higher pHs increased the stability of the particles. Zeta-potential of -33±1 mV improved the particles stability for three weeks at 4°C. The particles were spherical in shape ranging from 207 to 222nm in size. Low polydispersity index (PDI) values (0.2 to 0.3) indicated the particles had an acceptable homogeneity which is important in particle biodistribution. Incubation Caco-2 cells with a high nanoparticle concentration (2.5mg/ml) for 24h resulted in ~100% cell viability which revealed that the particles were not toxic. The release study showed that the particles were relatively resistant to pepsin and low pH in the gastric, but slowly released the encapsulated compounds (brilliant blue and β-carotene) in simulated intestinal conditions. The results suggested the prepared calcium-induced cruciferin nanoparticles are a promising delivery system to protect heat-sensitive bioactive compounds in the gastric.

Recovering Canola Protein and Other Products.

J.P.D. Wanasundara, T. McIntosh, and E.K. Mupondwa, Agriculture and Agri-Food Canada, Canada.

Canola (*Brassica napus*) non-oil fraction contains 38-40% protein predominantly composed of 11S cruciferin and 2S napin that differ in molecular size, structural organization, physico-chemical properties and biological activities. Separation of these proteins enables to utilize their unique properties therefore to maximize value of canola non-oil fraction. Selective solubility of napin in low pH allows this separation leaving cruciferin mixed with the fibre fraction. Napin can be recovered over >90% purity by membrane filtration. Cruciferin of napin-free

meal can be concentrated by releasing soluble fibre components using multi-active carbohydrases and this process generates fractions rich in soluble sugars and seed coat fibre. Alternatively, cruciferin can be isolated from napin-free meal by alkali extraction and subsequent concentrating steps simultaneously generating a canola seed fibre fraction. The napin-rich or cruciferin-rich protein products contain only one type of protein therefore can provide functions inherent to each protein. This approach of canola protein fractionation is significant for developing protein ingredients free of allergenic napin protein. The complete process tested in pilot scale ensured 78 to 80% protein capture on dry matter basis. The process, properties of protein products and economic analysis of scaled up process will be discussed.

Rice Protein: A Valuable Source for Nutrition. J. Wu, Y. Zou, B. Gong, and X. Xu*, Wilmar Global R&D Center, China.

Rice protein, including rice endosperm protein (REP) and rice bran protein (RBP), has been found to be a high-quality protein source with nutritional value and nutraceutical properties. It is a hypoallergenic food ingredient that could be used in infant formulations. Among cereal, rice protein has high digestibility and balanced amino acids profile, which can fulfill the amino acid requirements for 2-5 years old children. In addition, rice protein has many nutraceutical properties, such as hypocholesterolemic, antioxidant and anti-cancer activity. As a superior plant protein, rice protein could be an alternative to the animal or conventional plant protein, such as casein, soy and whey protein in selected food products.

Rice protein can be derived from the abundant and cheap agricultural by-products, such as rice bran, broken rice, chalky rice and stocked rice, which are mostly used as low-cost animal feed. The extractions of rice protein include alkali extraction, enzyme extraction, physical treatment, subcritical water extraction and their combinations. However, it remains a challenge for the commercial production of RBP due to its complex nature. So the efficient and economically viable method for RBP extraction needs to be further explored.

An Environmental Friendly Method for Extraction of Soybean Oil and Protein. X. Sui^{1,2}, B. Qi¹, Z. Wang¹, Y. Li¹, and L. Jiang¹, ¹Northeast Agricultural University, China, ²National University of Singapore, Singapore.

Commercial oils today are always extracted using organic solvent. However, the organic solvents are known to have environmental and safety problems, and, due to the increased awareness of safety and environmental issues, it is necessary to develop alternative methods to organic solvent extraction. The enzymatic aqueous extraction processing method as an alternative approach to the conventional organic solvent extraction method has been widely applied to extract oil and as well as protein from several seeds. This presentation will give updates on this environmental friendly method.

Surfactant-based Corn Oil Extraction Aids for the Dry-grind Ethanol Process. S. Lewis, Solenis, USA.

Ethanol derived from corn is a significant component of automotive fuel consumed in the United States. Most of the 13 to 14 billion gallons of annual fuel ethanol production comes from the dry-grind ethanol process. The evolution of the dry-grind industry has led to the production of increasingly diverse and more valuable co-products, including distillers corn oil (DCO). Approximately 80% of the industry produces DCO, with the majority being extracted from the condensed distillers solubles (CDS) in the “back end” (i.e., post-fermentation) of these bioethanol plants. The most widely-used method of separating DCO from CDS is through the use of a centrifuge, with typical yields of 0.2 to 0.5lbs DCO per bushel corn. This yield can be increased by 0.1 to 0.5lbs DCO per bushel corn through the addition of small amounts of surfactant-based corn oil extraction (COE) aids to the CDS prior to centrifugation, representing an additional \$1 to \$4 million in annual revenue for a typical 100 MMGY bioethanol plant. Although elucidating the exact mechanisms of increased oil yield is not trivial, several researchers have investigated the use of surfactants for the separation of vegetable oils from similar matrices. These fundamental studies provide insight into how COE aids may increase DCO yields.

A Comparison of Distillers Corn Oil and Distillers Milo Oil for Biofuels and Animal Feed Applications.

R.A. Moreau, USDA, ARS, ERRC, USA.

In recent years the number of dry grind ethanol plants in the US has risen to and plateaued at about 200 facilities. Currently, more than 90% of those

facilities are producing both ethanol and “back end” Distillers Corn Oil as a valuable coproduct. About half of the US Distillers Corn Oil (900 million pounds in 2013, compared to 2400 million pounds of conventional corn oil) is oil is being used as a biodiesel feedstock and about half is being used as a high-value poultry feed ingredient. Grain sorghum (milo) is currently being used as a feedstock for about 3% of the US fuel ethanol production. Now that a pathway from grain sorghum to an Advanced Biofuel has been approved by the EPA, many of the US dry grind ethanol plants are considering increasing their use of grain sorghum. Our laboratory is conducting research to understand the chemical composition of Distillers Milo Oil, and understand how it compares with Distillers Corn Oil, as a biodiesel feedstock, as a high-value animal feed, and as a source of unique waxes and for industrial applications.

Characterization of Condensed Distillers Solubles and Its Fractions for Composition of Main Nutrients, Minerals, and Amino Acids. K. Liu, USDA, ARS, USA.

In a previous report (J. Agric. Food Chem. 2013, 61:7325-7332), three methods were described to fractionate condensed distillers solubles (CDS) into several co-products (protein, oil, mineral, protein-mineral, glycerol-mineral, and/or glycerol fractions). For the present study, these new fractions were further characterized for main constituents, individual minerals and amino acid composition. Recovery of mass and main constituents was also investigated. Results show that mass recovery varied with fraction type, with the majority going to the protein or protein-mineral fraction. Protein, ash, and glycerol were mostly recovered into their respective fractions. CDS and its fractions contained 6 major minerals (Ca, Mg, P, K, Na, and S) and 4 minor ones (Cu, Fe, Mn, and Zn). When expressed on dry matter basis, both chemical and physical treatments caused significant mineral reduction in protein and glycerol fractions by shifting most minerals into mineral or mineral-enriched fractions. Amino acid composition, when expressed as % relative to total amino acids, differed significantly among fractions but the extent was not substantial for protein-rich fractions. These changes are favorable for improving end uses of the new co-products.

Utilization of Co-products Derived from the Corn Milling Industry in Poultry and Swine. B. Kerr¹ and G. Shurson², ¹USDA, ARS, NLAE, USA, ²University of Minnesota, USA.

Various co-products from the corn-milling industry have been fed to livestock for more than a century, with the acceptance of these feedstuffs coinciding with the advancement of our nutritional knowledge and their growing supply. The expansion of the ethanol industry has generated a variety of co-products, which due to availability and price, have become available for use as a potential feedstuff for poultry and swine. Most recently, ethanol companies have been extracting a portion of the oil from distillers dried grains with solubles (DDGS) resulting in a product called reduced oil-DDGS as well as the extracted oil called distillers corn oil (DCO). Distillers dried grains with solubles serve as an energy, amino acid, and phosphorus source in animal feeds while DCO serves as a concentrated energy source. The relative value of DDGS and DCO varies by livestock species according to their nutritional needs and their digestive capability, as well as the price differential among other feed ingredients. As ethanol production technology continues to evolve, so will the composition and diversity of co-products resulting from these processes. The impact of these technologies on the nutritive value of these co-products for swine and poultry has recently been evaluated and will be discussed in this presentation.

***In situ* Esterification of Camelina Oilseed Meal and Subsequent Recovery of Esters and Proteins.** T. Withana-Gamage, U. Wanasundara, and R. Green, POS BioSciences, Canada.

Crucifer oilseeds, such as rapeseed/canola and mustard, play a crucial role in the Canadian economy. In addition to providing oil, oilseeds accumulate considerable levels of proteins. Camelina (*Camelina sativa*) for example, a crucifer, contains 30-38% (w/w) oil and 30-34% (w/w) storage proteins in their seeds. The mucilaginous exo-layer of camelina seeds was first removed using a carbohydrase enzyme and washed multiple times to remove the mucilage. The de-mucilaged seeds were then dried to <5% moisture and subjected to base

catalyzed *in-situ* trans-esterification to produce fatty acid ethyl esters. The resulting Fatty Acid Ethyl Ester (FAEE) fraction was recovered by centrifugation and protein was recovered from the defatted meal by isoelectric precipitation. The applied processing scheme resulted in conversion of 96% of the triacylglycerol fatty acids into FAEE and the recovered FAEE was 95% purity. A protein concentration of 70% (w/w) was recovered in the protein-rich fraction. These results indicated that the developed *in-situ* transesterification method was effective for the direct conversion of Camelina fatty acids into FAEE and an enriched protein fraction was effectively recovered from the resulting de-fatted meal. Further scale-up of the developed method holds potential for increasing the economic value of Camelina.

Lipid Producing Sugarcane: Feedstock for Biodiesel Production. H. Huang, S. Long, and V. Singh*, University of Illinois at Urbana-Champaign, USA.

Biodiesel production from vegetable oils and animal fats has progressively increased over the past two decades. However, the limited supply of animal fats and the low amounts of oil produced per hectare from temperature seed oil crops, opportunities for further increases in N. America are limited. Genetically modified lipid producing sugarcane (lipid-cane) provides a great potential to produce biodiesel as an alternative feedstock, due to its much higher productivity versus soybean and canola. In this study, technoeconomic analysis models were developed for biodiesel and ethanol coproduction from lipid-cane assuming 2, 5, 10 and 20% lipid content of the harvested stem (dry mass basis). The models were compared to the conventional soybean biodiesel process model to assess its competitiveness in the market. With the increase of the lipid content in lipid-cane from 2% to 20%, the biodiesel production costs decreased from \$1.05/L to \$0.72/L (\$3.96/gal to \$2.73/gal), which are lower than the production cost from soybean at \$1.07/L (\$4.03/gal). Due to its high productivity, lipid-cane with 20% lipid content can produce 6,700L of biodiesel from each hectare of the land use, while soybean can only produce about 500L of biodiesel from each hectare of the land use.

PCP 5: Proteins and Peptides in Nutraceuticals: Functionality and Applications

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

Characterization of Functional Protein in Rice. H. Kumagai¹, S. Ina¹, A. Hase¹, T. Ando¹, M. Akao¹, and H. Kumagai², ¹Nihon University, Japan, ²Kyoritsu Women's University, Japan.

Rice albumin fraction contains unique protein (RA) that is indigestible by mammalian digestive enzymes. Previous work in our research group showed that RA can suppress postprandial hyperglycemia even on glucose loading. However, its mechanism of action has not been clarified yet. In addition, the high processing properties such as solubility, heat stability, foaming and emulsifying properties are important in order to use RA in various food products. The present study examined if the suppressive effect of RA on hyperglycemia is attributed to its indigestibility and if RA possesses desirable processing properties for food production. RA was extracted from rice flour with water and purified by ammonium-sulfate precipitation. It was hydrolyzed by trypsin after alkaline treatment to prepare rice albumin hydrolysate (RAH). Then, intraperitoneal glucose tolerance test (IGTT) for RA and oral glucose tolerance test (OGTT) for RAH were conducted. RA did not affect the blood glucose level in IGTT, while RAH suppressed the elevation of blood glucose level in OGTT. These results indicate that some peptides derived from RA possess a function to inhibit absorption of glucose from the small intestine. As for processing properties, RA retained high solubility in water even after heating, and showed high foaming and emulsifying properties at various pH.

Physicochemical Properties and Angiotensin-I Converting Enzyme Inhibitory Activity of Soy Protein Hydrolysates from a Non-genetically Modified Cultivar. Q. Nguyen, N. Hettiarachchy*, S. Rayaprolu, S. Kumar, S. Jayanthi, and P. Chen, University of Arkansas, USA.

Optimized Alcalase mediated hydrolysis was conducted to produce protein hydrolysates from soybeans with high protein yield, acceptable bitterness and clarity for beverage applications. The degree of hydrolysis ranged between 14 and 52% during the study. Soluble protein recovery ranged from 21% to 53% with decrease in turbidity and increase in surface hydrophobicity (S₀) which is correlated to bitterness of SPH treated with 1.0AU

(3.2μL/g) of Alcalase 2.4L. The SDS-PAGE analysis showed hydrolysis pattern in which 7S globulin and the two acidic sub-units of 11S globulin were hydrolyzed extensively in comparison to the two basic sub-units of 11S globulin. Limited enzymatic hydrolysis produced smaller molecular weight (MW) peptides - <17000Da. Among these SPHs, the one derived after 120min incubation had the highest soluble protein yield (43%), low S₀ value (35.4), low turbidity (0.88), and highest Angiotensin-I Converting Enzyme (ACE) inhibition activity (66.6%). This hydrolysate has potential use as protein rich nutraceutical for developing many non-GMO food product applications.

Effect of pH on the Physicochemical, Interfacial, and Emulsifying Properties of Pea, Soy, Lentil, and Canola Protein Isolates. C. Chang, S. Tu, S. Ghosh*, and M. Nickerson, University of Saskatchewan, Canada.

The physicochemical, interfacial and emulsifying properties of pea (PPI), soy (SPI), lentil (LPI) and canola (CPI) protein isolates at various pH values (3.0, 5.0, and 7.0) were investigated. Specifically, surface charge, solubility, surface hydrophobicity, interfacial tension, interfacial rheology, droplet size distribution, and emulsion stability of protein isolates were tested in response to changes in pH. Overall, all of the protein isolates showed better emulsifying abilities (smaller droplet size) and greater stability at pH 3.0, followed by them at pH 7.0 and pH 5.0. Isolates also had significantly higher solubility and surface hydrophobicity at pH 3.0. The interfacial tension, which was positively and negatively affected by the solubility and surface hydrophobicity, respectively, was positively correlated with interfacial rheology. Both droplet size and emulsion stability were further negatively influenced by the interfacial tension and interfacial rheology, respectively. Moreover, CPI and LPI showed better emulsifying ability and interfacial rheological properties (larger complex modulus) than SPI and PPI. Both of them also had higher solubility and surface hydrophobicity. The results greatly extend the knowledge to understand emulsifying characteristics of plant proteins.

Physical, Chemical, and Structural Changes of WPI Induced by Maillard Reaction with Pectin in Dry State. P.X. Qi¹ and Y. Xiao^{1,2}, ¹USDA, ARS, ERRC, USA, ²Zhejiang Academy of Agricultural Sciences, China.

We investigate the physical, chemical and structural changes of whey protein isolate (WPI) that occurred upon reacting with sugar beet pectin (SBP) by dry heating at varying weight ratios (1:0.2, 1:0.4 and 1:1), controlled temperature (60°C) and relative humidity (79%). Total and protein solubility of the resulting products were determined by weight and the Bradford assay method respectively. Results showed an increase in both total solubility and protein solubility in the conjugates compared to the WPI control sample. Chemical changes of the conjugates including free SH and NH₂ contents were determined by DTNB and ninhydrin assays, and only displayed a noticeable decrease (~15%) at the highest pectin level (1:1). Secondary structural changes were measured by FTIR and far-UV circular dichroism (CD) spectroscopies and only showed minimal alteration. Tertiary structures of the conjugates were characterized by near-UV CD and intrinsic fluorescence, and demonstrated disruptions in the tryptophan residue environment compared to WPI. Thermal stability of the conjugates was also studied by CD and fluorescence, and showed an increased level of heat resistance than WPI. This work established an optimal weight ratio for forming conjugates between WPI and SBP with improved physical properties while causing limited structural changes.

Purified Single Peptides from Soybeans Show Inhibitory Activity Against Human Blood and Colon Cancer Cells. S. Rayaprolu, N. Hettiarachchy*, and P. Chen, University of Arkansas, USA.

Protein hydrolysates were prepared by proteolysis with alcalase enzyme using response surface methodology. These hydrolysates were tested for resistance against simulated gastrointestinal juice, fractionated into definite molecular size fractions and tested for anticancer activity. Protein fractions between 10 and 50kDa molecular size cut-offs showed highest activity which were purified using peptide specific HPLC affinity column. The pure peptides were tested for anti-cancer activity against colon cancer cell line - HCT-116 and blood cancer cell line - CCRF-CEM with (3-(4, 5-dimethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell titer assay. A known anti-cancer agent, Genistein, was used to compare the anti-cancer activity. The highest

anti-cancer activity of approximately 78% by a single peptide was observed against blood cancer cells, which was significant. The impact of this study lies in utilizing an underutilized and inexpensive co-/by-product to generate nutraceutical anti-cancer peptides. Purified and characterized single peptides could be potential gastrointestinal-environment friendly ingredients with health benefits particularly against cancer, which is known to have a high mortality rate.

Nutraceutical Applications of Peptide Aggregates in Plastein. C.C. Udenigwe, Dalhousie University, Canada.

Proteases are known to induce peptide aggregation to form gels or thixotropic products known as plastein. Previous studies have demonstrated the prospects of plastein in enhancing the nutritional quality of proteins and debittering protein hydrolysates, but these properties are yet to be commercially applied in food product development. Plastein was thought to be formed by peptide condensation and transpeptidation, but recent findings suggest that the process is entropy-driven mostly by physical interaction of aggregating peptides held together by predominantly hydrophobic bonds. Emerging findings have demonstrated that the peptides within some plastein display better bioactivity compared to their hydrolysate precursors, including antioxidative, antihypertensive, antithrombotic and bile acid-binding properties. Plastein is stable at pH 2-11 and at 100°C. These properties enhance the prospective applications of the aggregates in functional food development considering their positive impact on the sensory properties of protein hydrolysates. However, aggregation does not improve the stability of the peptides to cleavage by gastrointestinal proteases. The specific role of the enzymes in inducing plastein formation remains unclear; if known, this can facilitate the design and development of highly functional plastein for nutraceutical applications.

Long-term Stability of Mixed Dairy and Pea Protein-stabilized Nanoemulsions. M. Yerramilli and S. Ghosh, University of Saskatchewan, Canada.

In this research, pea protein isolate (PPI) was used to partially to replace casein sodium salt (CSS) in order to investigate its efficacy in the formation and long-term stabilization of nanoemulsions. 5wt% oil-in-water nanoemulsions were prepared by both CSS and PPI and their 1:1 mixtures with a total

aqueous-protein concentration of 5, 7.5 and 10wt%. All CSS nanoemulsions (average droplet size < 200nm) displayed weak re-dispersible creaming behaviour (due to depletion flocculation by excess proteins). In contrast, PPI failed to produce nanoemulsions (> 200nm) displaying excessive droplet and protein aggregation. Interestingly, the mixed CSS and PPI-stabilized nanoemulsion did not display any creaming or aggregation. Although these nanoemulsions displayed PPI sedimentation over one month period (indication of un-adsorbed PPI in the aqueous phase), they remained stable throughout the experimental timeframe with average droplet size in the range of 190-220nm. It was proposed that the presence of excess unabsorbed PPI promoted depletion stabilization towards CSS-stabilized droplets thereby preventing both the destabilizing mechanisms seen in individual protein-stabilized nanoemulsions. The mixed-protein stabilization could be a novel way to utilize plant proteins in the development of nanoemulsions.

Antioxidant Effects of a Hemp Seed Protein Hydrolysate During Long-term Oral Administration to Spontaneously Hypertensive Rats. R.E. Aluko, University of Manitoba, Canada.

The aim of this work was to determine the ability of a hemp seed protein hydrolysate (HPH) to reduce oxidative stress factors while increasing antioxidant factors during 8-12 weeks of oral administration to spontaneously hypertensive rats (SHRs). The antioxidant HPH was produced through simulated gastrointestinal in vitro hydrolysis of defatted hemp seed meal, which consisted of consecutive treatments with pepsin and pancreatin. Young (8 weeks old) SHRs were divided into 3 groups (8 rats/group) and fed diets that contained 0.0, 0.5, or 1.0% (w/w) HPH for 8 weeks after which half of the rats were sacrificed for blood collection. The remaining rats (now categorized as 'old') underwent a 4-week washout period followed by additional 4 weeks of feeding before they were sacrificed for blood collection. The plasma was analyzed for total antioxidant capacity (TAC) and superoxide dismutase (SOD), catalase (CAT) and total peroxides (TPx). Results showed that plasma TAC, CAT and SOD levels were significantly ($p < 0.05$) lower in the old SHRs when compared to the young SHRs. Addition of HPH to the diets resulted in significant ($p < 0.05$) increases in plasma SOD and CAT levels in both the young and old SHRs groups while TPx levels were decreased.

PCP-P: Protein and Co-Products Poster Session

This session is sponsored in part by DuPont Nutrition & Health

Chairs: M.P. Hojilla-Evangelista, USDA, ARS, NCAUR, USA; K.A. Campbell, DuPont Health & Nutrition, USA; and P.X. Qi, USDA, ARS, ERRC, USA

1. Comparative NMR Investigation of Cottonseed Protein Isolate and Soy Protein Isolate. Z. He¹, J. Zhong², and H. Cheng¹, ¹USDA, ARS, SRRRC, USA, ²Intertek Analytical Services, USA.

Currently, cottonseed protein isolate (CSPI) is not regarded as much as important as soy protein isolate (SPI) in the term of industrial raw materials. Increasing knowledge of its structure would be helpful in promotion of CSPI's wider industrial application. In this study, we characterized the protein structures of both CSPa and SPI by multiple ¹³C nuclear magnetic resonance (NMR) spectroscopic techniques. ¹³C cross polarization (CP)/ magic angle spinning (MAS) and single pulse excitation (SPE)/MAS spectra were obtained and deconvolution on their peaks was performed to get quantitative information of different C functional groups. Dipolar Dephasing (DD)-MAS technique was used to identify weakly coupled and strongly coupled carbons in the two proteins structures. Time domain (TD) NMR was performed to calculate the ¹H free induction decay (FID) relaxation time (T₂) data. The T₂ data were further resolved into different T₂ components, corresponding to rigid, (intermediate) and mobile domains of the protein structures. The difference and similarity in these spectral features between CSPI and SPI are under analysis.

2. Proteins and Blood Flocculate Lignin. G.J. Piazza¹, J.H. Lora², and R.A. Garcia¹, ¹USDA, ARS, ERRC, BOAC, USA, ²GreenValue Enterprises LLC, USA.

Non-sulfonated lignin, a co-product of biomass conversion to cellulose pulp and fuel ethanol, is a renewable material which has experienced rapid supply growth. Lignin flocculation could aid the purification of lignin and also be used to remove lignin in wastewater. There has been little research on flocculation of non-sulfonated lignin. Potential lignin flocculants were tested on fine suspensions of highly purified lignin. Synthetic polymeric cationic flocculants, poly(diallyldimethylammonium chloride), polyquaternaryamine, and high molecular weight cationic polyacrylamide, were found to be effective lignin flocculants. Unfortunately, this type of flocculant is toxic to aquatic life. Neutral poly(ethylene oxide) and iron (III) sulfate were also able to flocculate lignin, although their use may

cause undesirable environmental consequences. Bovine hemoglobin (HEM), wheat gliadin, and porcine blood were effective lignin flocculants. Chemical oxygen demand (COD) measurements were much lower with HEM than with gliadin because gliadin was solubilized in ethanol/water which greatly increased COD levels. HEM flocculation was optimal at pH 4.8-6.0 and diminished at higher pH values. Substances with no flocculant activity: anionic polyacrylamide/calcium chloride, neutral polyacrylamide, and soybean protein. Zeta potential measurements aided the interpretation of flocculant action.

3. Comparison of the Adhesive Properties of Sequentially Extracted Water- and Alkali-soluble Fractions of Cottonseed Protein. Z. He and D. Chapital, USDA, ARS, SRRRC, USA.

The interest in oilseed protein isolates as wood adhesives has resurrected recently, as these plant raw materials are considered renewable and environmentally-friendly. Oilseed protein isolates comprise of multiple fractions with different physico-chemical properties. In this work, we sequentially separated cottonseed protein into a water-soluble (CSPw) and an alkali-soluble (CSPa) fraction. Fluorescence spectral characterization revealed that CSPw is more hydrophilic whereas CSPa tends to be hydrophobic. We tested the adhesive properties of these two fractions on maple wood veneers. The wood adhesives were made by mixing CSPw or CSPa (1.2g) into water (100g) without pH changes or with pH adjusted to 11. The adhesive strength and water resistance of the protein fractions were evaluated by the shear strength of the bonded wood pairs at break with or without water soaking, respectively. The chemical and amino acid composition of CSPw and CSPa is under analysis. Statistic analysis will be conducted to find if there is any correlation between the adhesive behaviors and structures/composition of the two proteins.

4. Kinetics of Enzyme Inhibition and Blood Pressure Lowering Effects of Salmon (*Salmo salar*) Protein-derived Peptides *in vitro* and in Spontaneously Hypertensive Rats. I.D. Nwachukwu¹, A.T. Girgih¹, and R.E. Aluko^{1,2}, ¹University of Manitoba, Canada, ²Richardson Centre for

Salmon protein hydrolysate (SPH) produced from enzymatic hydrolysis using a combination of gastrointestinal enzymes (pepsin, trypsin and chymotrypsin) and its RP-HPLC peptide fraction (SF3) significantly ($p < 0.05$) inhibited the activities of angiotensin I-converting enzyme (ACE) by 90 and 92% respectively, as well as the activities of renin (52 and 72% respectively). This result was validated by the higher IC_{50} values exhibited for renin (0.14 and 0.26mg/mL) compared to those of ACE inhibition (0.09 and 0.11mg/mL). SPH and SF3 showed both uncompetitive and non-competitive modes of inhibition of ACE and renin enzymes respectively as indicated by associated decreases in V_{max} , K_m and enzyme catalytic efficiency (CE) in the presence of the inhibitors. *In vivo* blood pressure-lowering tests using spontaneously hypertensive rats (SHRs) as a test model indicated that SF3 significantly reduced systolic blood pressure by up to -42.1 ± 3.380 mmHg, a value slightly higher than the maximum lowering effect (-40.45 ± 1.62 mmHg) attained by the drug captopril, which was used as positive control.

5. Cutaneous Permeability of Deamidated and/or Hydrolyzed Wheat Gliadin. N. Matsukaze¹, R. Abe¹, M. Akao¹, H. Kumagai², and H. Kumagai¹, ¹Nihon University, Japan, ²Kyoritsu Women's University, Japan.

HCl-treated wheat protein (HWP) is often used in various products such as cosmetics and shampoo. Recently, immediate hypersensitivity to HWP contained in soap was reported, but the mechanism of this allergic sensitization has not been fully clarified yet. HCl treatment causes not only hydrolysis but also deamidation. This study was conducted to examine the effect of hydrolysis, deamidation and presence of a surfactant on cutaneous permeability of allergen in order to specify the responsible factor for sensitization. Gliadin, the major allergen in wheat protein, was extracted from gluten using 60% ethanol. Hydrolyzed deamidated gliadin (HDG) was prepared by HCl, hydrolyzed gliadin (HG) was by pepsin, and deamidated gliadin (DG) was by cation-exchange resins. These gliadin samples were applied onto porcine skin with and without SDS, and the level of

allergen permeated through the skin was measured by inhibition ELISA using an antibody prepared by HWP.

Cutaneous permeability of HDG was almost the same as that of HG and DG in the absence of SDS. On the other hand, permeability of allergen increased in the presence of SDS. These results indicate that the presence of surfactant would be the most crucial factor for cutaneous sensitization to wheat protein.

6. Proteins of *Camelina sativa* (L.) Crantz Oilseed—Investigation of Protein Types and Their Structure. S. Perera^{1,2}, T. McIntosh¹, R. Tyler², and J.P.D. Wanasundara^{*1,2}, ¹Agriculture and Agri-Food Canada, Canada, ²University of Saskatchewan, Canada.

Camelina sativa (L.) Crantz or false flax is a crucifer developed as an industrial oilseed crop for prairie conditions. The oil-free meal of camelina contains 40-43% protein and can be used in animal feed and protein-based product development. Information on camelina seed proteins is scarce, and limited to amino acid composition of meal protein. This study investigated camelina seed storage proteins in order to understand their structural and physico-chemical characteristics. Seeds of a double-haploid line (DH55) produced under greenhouse conditions was studied. Seed coat mucilage removal followed by de-oiling was employed to prepare seed meal for protein extraction. Protein extracted at pH 8.5 was separated and purified to obtain large, neutral (11S cruciferin) and small, basic (2S napin) proteins. Both these proteins were obtained >95% purity employing chromatographic processes. Protein identity was confirmed by 1D and 2-D electrophoresis combined with mass spectroscopy. Details of protein structure were obtained by FT-IR, Circular Dichroism (far and near UV), and fluorescence spectroscopy. Thermal properties of the proteins were also examined. All these investigations indicate camelina seed protein fraction predominantly contains proteins with characteristics similar to cruciferin and napin in other crucifers as canola.

7. Impact of Industry-relevant Heat Processing on Navy Bean Proteins Profile, Structural, and Immunoreactive Properties. L. L'Hocine, M. Pitre, S. Villeneuve, A. Achouri, and L.P. Des Marchais, Agriculture and Agri-Food Canada, Canada.

Pulses are gaining momentum as healthy food choices and the pulse industry has been actively searching for novel application strategies, such as

processing pulses into flours or protein isolates for incorporation into different food systems. It is therefore crucial to evaluate potentially adverse effects associated with the novel processing of pulse grains. Our objective was therefore to assess the impact of heat processing on bean protein profile, structural and allergenic properties. Navy beans were subjected to various wet or dry heat treatments, grinded into flours, and extracted. Extracts were analysed by gel electrophoresis and Fourier transform infrared spectroscopy (FTIR). Changes in protein immunoreactivity were assessed by IgE- immunoblotting and Inhibition-ELISA. Results demonstrated that the type of heat processing has a major impact of bean protein profile, structure and immunoreactivity. Boiling resulted in a considerable loss and degradation of soluble proteins with a subsequent reduction in IgE-binding, while roasting led to an increased IgE-binding with the appearance of new immunoreactive polypeptides bands. These differences in immunoreactivity could be related to the important differences in processing-induced changes in protein secondary structure as revealed by FTIR analysis.

8. Simultaneous Adsorption of Sodium Caseinate and Pea Protein Isolate at the Oil Droplet Surface of Nanoemulsions. M. Yerramilli and S. Ghosh, University of Saskatchewan, Canada.

In this research, the potential use of pea protein isolate (PPI) as an emulsifying agent was investigated in combination with casein sodium salt (CSS), for the development of nanoemulsions (NEs). Oil-in-water NEs were prepared with 5wt% oil and various concentration of 1:1 mixture of CSS and PPI using high-pressure homogenization. NEs stabilized by similar concentrations of PPI and CSS alone were also prepared as control. Single protein-stabilized NEs failed to display long-term stability and fluid flow behaviour over 2 months, but those stabilized by mixed protein remained stable. Interfacial protein composition (surface load) was quantified after separating the cream and serum layer and determining the total protein concentration in the serum layer. In NEs stabilized by individual proteins, the protein surface load was highest for PPI and least for CSS. Interestingly, surface load for mixed NEs ranges between the two individual protein-stabilized NEs with values closer towards CSS, indicating partial replacement of CSS with PPI at the oil droplet surface. SDS-PAGE analysis of interfacial and serum layer confirmed presence of vicilin fraction of PPI at the interface along with CSS. Hence

PPI could successfully replace CSS in NE formation and improving long-term stabilization.

9. Extraction and Properties of Dried Alfalfa Leaf Protein. M.P. Hojilla-Evangelista, G.W. Selling, P.J. Weimer, and R. Hatfield, USDA, ARS, USA.

Alfalfa, traditionally used for animal feed, has attracted attention as potential feedstock for biofuels and co-products from processing need to be assessed for value-added uses. This study describes extraction of protein from dried alfalfa leaves and the functional properties of the protein. Ground, dried alfalfa leaves contained 26% db crude protein. Albumins were the major fraction (26% of total protein) while globulins were the least (1%). SDS-PAGE detected 9 polypeptide bands between 7-77 kDa. The method of alkali solubilization for 2hr at 50°C, acid precipitation, dialysis, and freeze-drying produced a protein concentrate (60% crude protein). Our alfalfa leaf protein concentrate showed only moderate solubility (maximum 50% from pH 5.5 to 10), but good foaming properties at pH 2 (117mL, 90% volume retention). The protein also showed high emulsifying activity (158-219m²/g protein) and emulsion stability (17-49min), with values increasing with pH. Alfalfa leaf protein was very stable to heating (1-2% loss of solubility) at pH \geq 7.0. Dried alfalfa leaves may not be suitable as starting material for production of protein concentrates, given the difficulty of achieving high yields and high-purity protein product. The protein would be still useful for nutritional value.

10. Improvements to the Gossypol HPLC Analytical Method. M.K. Dowd and S.M. Pelitire, USDA, ARS, SRRC, USA.

The AOCS method for determining isomeric gossypol (Recommended Practice Ba-8a 99) treats the sample with a chiral amine and heat to form a diastereomeric pair of gossypol-Schiff's base amines. The amine adducts are then separated on an octadecyl reverse-phase stationary phase and detected by UV adsorption. Over the years, we have made several modifications to this method. The revisions include a reduction of sample scale, which eliminates transfers, improves measurement repeatability, and lowers cost, and an altering of the detection wavelength, which improves sensitivity. Additionally, a few unstudied aspects of the method were evaluated. For example, the complexing reagent (containing the chiral amine) can be stored in the dark at -20°C for at least 6 months without significant degradation. Given that the chiral amine

is expensive, this storage method is preferable to the one week storage time recommended in the current practice. Post-reaction amine derivative stability was also evaluated. Storage in the dark at -20°C resulted in relatively slow degradation (at a rate of 0.3% per day) but storage at room temperature under laboratory fluorescence lights resulted in faster degradation (at a rate of 6% per day).

11. Foaming Characteristics of Lentil Legumin-like Protein as Affected by pH Relationship with Molecular Structure, Shear, and Dilatational Rheology. M. Jarpa-Parra¹, F. Bamdad¹, Z. Tian¹, H. Zeng¹, J. Han², and L. Chen¹, ¹University of Alberta, Canada, ²Alberta Agriculture and Rural Development, Canada.

Lentil legumin-like protein interfacial and foaming properties were investigated in relation to its molecular structures under different pH using a combination of FTIR, light scattering, shear and dilatational rheology. The results revealed protein dissociation into 3S form at pH 3, while at pH 7 remains as 13S polypeptides and small aggregates. Higher surface hydrophobicity and smaller hydrodynamic size at pH 3 might explain high diffusion and adsorption rates, while favourable interfacial conformational changes for pH 5 and 7 can be related to high re-arrangement coefficients. Molecular characterizations and surface activity study revealed that fast diffusion along with high surface hydrophobicity and solubility were responsible for high foaming capacity. Dilatational and shear rheology results supported high stability for solutions at pH 7 (84min). Combination of α -helix presence, medium molecular size, and solubility/hydrophobicity balance helped to build strong inter-protein networks at the interface. Also, protein at pH 5 has a good foaming capacity and stability (51min), due to the rapid increase of elasticity modulus and interfacial multilayer formed by protein aggregation. Such foaming capacity and foam mean life at this pH range open opportunities for food applications where long-term stability is sought.

12. Foaming Performance of N-Acylated Peptides Recovered from Specified Risk Material. P. Appadu, M. Chae, L. Mercier, and D. Bressler, University of Alberta, Canada.

Several thousand tonnes of specified risk material (SRM) are landfilled annually at considerable economic and environmental costs due to feed bans instituted by the Canadian Food

Inspection Agency (CFIA). SRM refers to the tissues in cattle where misfolded proteins that cause bovine spongiform encephalopathy, or “mad cow disease”, most likely concentrate. This research demonstrates a novel technology platform for the conversion of the peptides isolated from thermally hydrolysed SRM into industrial foaming agents. SRM was hydrolysed by a method approved by the CFIA using subcritical water (180°C, >173psi). The recovered peptides (>85%) were acylated with hexanoyl chloride (2 eq/-NH₂ group) and the surface and micellar properties, such as foam stability, water retention, and surface tension, were evaluated at various pH. The grafting of a six-carbon chain onto the peptides improved the foam stability and water retention when compared to the unmodified peptides. For example, at time = 0mins and pH 7.0, the foam volume and water retention improved by 10% and 142% respectively while the surface tension increased from 28 to 35mN/m. The addition of longer hydrocarbon chains is expected to further enhance the foaming performance and aid in the development of biodegradable fire-extinguisher foams.

13. Sodium Deoxycholate-binding Affinity of a Hydrophobic Casein Plastein. A. Mohan and C.C. Udenigwe, Dalhousie University, Canada.

Peptide aggregation during plastein formation can alter their structures, surface properties and bioactivities. In this study, isolated casein plastein (CPI) was found to increase the surface hydrophobicity and sodium deoxycholate (SDC)-binding activity of the original casein hydrolysate (CH) by 4- and 2-folds, respectively. The binding of 0.03-4mM SDC to CH, CPI, and the unisolated casein plastein (CPu) was fitted into one-site total binding curve to determine the ligand dissociation constant (Kd) and maximum specific ligand binding (Bmax). CPu had the highest Bmax (0.85mM/mg protein) followed by CH (0.64 mM/mg protein) and least for CPI (0.50mM/mg protein). However, CPI had the strongest affinity for SDC with the lowest Kd (0.74mM; >2 folds lower than the Kd of CH and CPu). Since plastein contains high amounts of hydrophobic amino acids, the low Kd in binding SDC can be attributed to the increased total and surface hydrophobicity of CPI, which can enhance its interaction with the ligand's hydrophobic core. Understanding deoxycholate-binding affinity of protein hydrolysates and the aggregates will enhance the design of functional ingredients for managing

hyperlipidemia and deoxycholate-induced colon cancer.

14. Effect of Graphene Oxide Preparation Conditions on Functionality of Canola Protein–Graphene Oxide Hybrid Wood Adhesive. N.P. Bandara (*Honored Student Award Winner*), Y. Esparza, and J. Wu, University of Alberta, Canada.

Canola is the second largest oil seed in the world, and oil processing generates a great deal of meal with a protein content ~ 35%. Developing alternate applications such as wood adhesives is expected to improve the profitability of the industry. Wood adhesives are mainly produced using petroleum byproducts. However, due to environmental concerns, industry is moving towards bio-based polymers. Protein adhesives have common drawbacks such as poor water resistance. The objective of this research is to develop canola protein (CP)–graphene oxide (GO) hybrid adhesive under different GO preparation conditions. GOs were analyzed for changes in composition, functional groups and thermal properties with XPS and DSC, related to their preparation conditions. Adhesion strength was measured using Instron tensile loading. Adhesive samples were characterized using XRD, FTIR, and DSC for GO dispersion, structural and thermal properties. Experiments were duplicated and results were analyzed using ANOVA. GOs prepared with different conditions exhibit changes in adhesion strength where lower C:O ratio exhibit higher strength. Reduction of denaturation temperature with GO prepared with longer oxidation time and changes in secondary structure were observed in CP-GO adhesives. GO were effective in developing adhesives with improved functionality.

15. Biopolymer-based Particles for Adsorption and Recovery of Oil in Emulsion Layer of Condensed Corn Distillers Solubles (CCDS). J. Wong and B.P. Lamsal*, Iowa State University, USA.

The use of biopolymers as adsorbent to enhance recovery of free oil from oil-in-water (O/W) emulsion is emerging as a potential innovative approach to traditional practices. The ability of biopolymers to adsorb oil are mainly due to physisorption due to *hydrophobic interactions* and *electrostatic interactions*, depending on environment. The goal of this study is to look into the possibility of using low-cost natural polymers- *chitosan*, *zein* and *lignin*- as adsorbent for recovery of free oil from O/W emulsion of corn condensed corn distillers solubles

(CCDS). Manufacture and performance of biopolymer particles will be optimized for polymer concentration, pH, and contact times, it is hypothesized that there will be a significant oil uptake by the respective biopolymers, and the adsorption efficiency will further increase with decreasing pH due to electrostatic forces, resulting in higher recovery of free oil. Langmuir and Freundlich adsorption models will be tested to describe the batch adsorption isotherms of bio-adsorbents, which will verify, the mechanism of oil adsorption to be physisorption.

16. Intermolecular Interaction and Formation of Coacervates of Bovine Serum Albumin with Flaxseed (*Linum usitatissimum* L.) Gum. J. Liu¹, Y.Y. Shim^{1,2}, Y. Wang³, and M.J.T. Reaney^{1,2,3}, ¹University of Saskatchewan, Canada, ²Prairie Tide Chemicals Inc., Canada, ³Jinan University, China.

The formation of bovine serum albumin (BSA) coacervates with flaxseed gum (FG) was investigated by turbidimetric analysis as a function of pH (6.0–1.4), biopolymer weight ratio ($R = 1:15$ – $15:1$, w/w), NaCl concentration (0–100mM), and urea concentration (0–150mM). Critical phase transition pHs (pH_c , pH_{ϕ_1} , and pH_{ϕ_2}) associated with coacervate formation between BSA and FG mixture ($R = 1:1$) were observed at 5.4, 4.8, and 2.0, respectively. The maximum interaction ($OD_{600} = 0.818 \pm 0.005$) was found at pH 4.0 (pH_{max}) and $R = 2:1$. As R increased from 1:15 to 15:1 the pH_{ϕ_1} and pH_{ϕ_2} increased from 4.2 to 5.2 and from 1.8 to 2.8, respectively. The shift of pH_{max} from 2.8 to 4.8 was consistent with the isoelectric point of BSA-FG mixtures, while the pH_c was independent of R . NaCl significantly decreased the pH_c , pH_{ϕ_1} , and pH_{max} , while the pH_{ϕ_2} was increased. An overall shift of turbidity curve towards to more acidic pH was observed in the presence of urea. Particle size distribution of BSA-FG at different pH provided further insight into the coacervates formation. Findings from this study will help to design new ingredients for food and biomaterial applications.

17. Conversion of Canola Meal into a High Protein Feed Additive by Submerged and Solid-state Fungal Incubation Processes. J.R. Croat¹, M. Berhow², B. Karki¹, K. Muthukumarappan¹, and W.R. Gibbons¹, ¹South Dakota State University, USA, ²USDA, ARS, NCAUR, USA.

Canola (*Brassica napus*) is grown widely in Canada and the northern US as a source of edible oil

or for biodiesel/jet fuel production. Globally, canola meal is second only to soybean meal as a protein source for livestock. A general limitation of *Brassica* spp. meals is the presence of glucosinolates (GLS). Canola was bred to contain lower levels of GLS and erucic acid, however feed inclusion rates are still limited to 25-30% of livestock diets. The objective of this research is to develop a microbial process to metabolize GLS and breakdown products into non-toxic components, to enable higher inclusion levels in livestock rations. An additional objective is to boost the protein levels and digestibility. Cold pressed and hexane extracted canola meals were processed using several metabolically diverse fungal cultures. *Aurobasidium pullulans*, *Trichoderma reesei*, *Fusarium venenatum*, *Pichia kudriavzevii*, and *Mucor circinelloides* were grown in submerged and solid-state culture at 10 and 50% solid loading rate, respectively. Samples were analyzed for protein, GLS, fiber, and residual sugars. Results have shown up to 23% improvement in protein and 98% reduction in GLS. Further research will optimize the conversion process using various pretreatment techniques and nitrogen supplementation to further increase protein content.

18. Flaxseed Orbitide Antibodies. P.D. Jadhav¹, Y.Y. Shim^{1,2}, and M.J.T. Reaney^{1,2,3}, ¹University of Saskatchewan, Canada, ²Prairie Tide Chemicals Inc., Canada, ³Jinan University, China.

Flaxseed (*Linum usitatissimum* L.) a widely consumed food has been associated with numerous health benefits. Flaxseed orbitides (cyclolinopeptides/CLs), having molecular weights of approximately 1kDa have been isolated from flax oil. Orbitides are circular peptides, linked *via* an N- to C-terminal peptide bond, composed of five to twelve proteinogenic amino acids. Flaxseed CLs were modified through methionine to introduce hydroxyl (OH) groups. The CL haptens were conjugated to bovine serum albumin (BSA) and these BSA hapten complexes were used to elicit polyclonal antibodies (pAbs) production in rabbits. Competitive indirect enzyme-linked immunosorbent assay (CI-ELISA) was developed by the use of specific pAbs and horseradish peroxidase conjugates. The pAbs can be used for the detection of CLs in flax, flaxseed samples, flaxseed containing foods, and the detection of CLs in tissue samples, wastes, and body fluids of animals fed flaxseed.

19. Yield, Polypeptide Composition and Protein Digestibility of Ferritin (Iron-binding Proteins) Isolated from Manitoba Legume Seeds. F.A. Gesinde and R.E. Aluko, University of Manitoba, Canada.

Treatment of iron-deficiency anemia (IDA) is currently through iron supplementation with ferrous salts but the WHO has recommended the use of more readily bioavailable forms of iron. This research work focuses on phytoferritins from Manitoba-grown legume seeds due to low cost and widespread availability of plant raw materials. The objective of this study is to produce a ferritin concentrate that can be used as an ingredient to formulate nutraceutical products (pills or tablets) for treatment of IDA. Different legume seeds were purchased in Winnipeg and a commercially produced pea protein isolate (PPI) was obtained from Nutri-Pea Ltd. Optimization of ferritin extraction from legume seeds and PPI was achieved using standard isolation and purification methods. Isolates were characterized for amino acid composition using analytical HPLC. Polypeptide composition was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). There were significant ($p < 0.05$) differences in ferritin protein contents (61.36%-74.28%), yield (4.9-19 g/100g and iron:protein ratio (0.034-0.82). Iron content varied significantly ($p < 0.05$) from 29.51 to 45.49 mg/100g, which tended to be directly correlated with level of acidic amino acids. SDS-PAGE of the ferritin isolates showed a main 50 kDa polypeptide band while FPLC chromatograms also indicated one dominant peak, which shifted to smaller sizes after protein digestion and confirmed susceptibility to digestion. This work demonstrates the feasibility of phytoferritin production from Manitoba pulses, which could serve as a better iron supplement than current inorganic forms.

20. Challenges and Prospectives of Transglutaminase Application in Flour-based Products. O.M. Shanina, T. Gavrish, I. Galyasniy, A. Teymurova; Petro Vasilenko Kharkiv National Technical University of Agriculture, Ukraine

Enzymes significantly increase a speed of technological processes, enhance an output and a quality of finished food product, allowing rational usage of valuable raw materials. Combination of commercial enzymes and different sources of food proteins is an effective method to enhance the properties of flour. Providing a certain dough structure and finished products' texture is important problem in production of gluten-free bread and

traditional pasta from flour raw materials with unstable quality. We developed novel methods of TG application in gluten-free baked and steamed bread technologies. These innovative technologies are based on a reactive action occurred between TG and proteins derived from meat and milk raw materials (e.g., whey, gelatin, different kinds of flours). Conducted studies showed the effectiveness of TG in a

combination with vegetable and animal proteins improving textural and organoleptical properties of gluten-free bread. As a technological solution we also suggest the combination of TG and flour derived from vegetable oil production by-product in order to increase nutritional value of traditional pasta, enhance rheological properties of the dough while using baking wheat flour.