PCP 1: Assessment of Protein Nutritional Quality: Digestibility and Bioavailability

Chairs: E. Krul, Solae LLC, USA; N. Deak, Solae LLC, USA; and J. Wanasundara, Agriculture & Agri-Food Canada, Canada

Human Requirements for Protein and Amino Acids: How Much Do We Really Know? D.J. Millward, DJ Millward, Twickenham, Middlesex, UK.

Although the assessment of protein nutritional quality can be done, at least in theory, with methods which measure its components directly, in practice quality is predicted as a function of its digestibility & indispensable amino acid (IAA) score. Scoring requires a reference IAA profile which in turn requires knowledge of both IAA & protein (PROT) requirements for the population of interest & the establishment of these values is inherently difficult. The currently accepted values were compiled from an extensive review by FAO/WHO (2007) & this report was only able to identify PROT & IAA requirements for healthy adults: i.e. maintenance requirements. For all other population groups these maintenance values were used in a factorial model of maintenance & growth to predict requirements. It is the case that none of the listed values for IAA & PROT requirements are entirely secure and in one case, i.e. pregnancy, the requirements may be unsafe. There is also considerable current debate about whether the current model based on minimal PROT & IAA requirements is appropriate & should be replaced with optimal requirements assumed by advocates to be higher values. This presentation will review the difficulties & uncertainties associated with the established human PROT & IAA requirements in the context of an adaptive metabolic demand nutritional model for dietary protein.

The Evolution of Protein Quality Evaluation. G.J. Hughes, DuPont Nutrition & Health, St. Louis, MO, USA.

It has long been recognized that both quantity and quality of dietary protein is important for human health. However, debate continues on the most appropriate way to assess protein quality for scientific as well as regulatory purposes. The Protein Efficiency Ratio (PER), a rat assay method, has been used for nearly 100 years. As more studies on human amino acid requirements and protein bioavailability became available, newer methods have been proposed. The Protein Digestibility-Corrected Amino Acid Score (PDCAAS), based on comparing the amino acid composition of a dietary protein to a reference amino acid profile and adjusting for protein digestibility, was introduced in 1991 and has been adopted by regulatory agencies globally. More recently, another method, the Digestible Indispensable Amino Acid Score (DIAAS) has been proposed, recommending different amino acid reference patterns and ileal digestibility to assess amino acid bioavailability. Another method under discussion is the Indicator Amino Acid Oxidation Method. These newer methods are not generally accepted and lack regulatory adoption. It is clear that researchers are seeking to improve protein quality assessment for human nutrition but it appears that more work needs to be done to determine the most biologically relevant, reproducible, and cost-effective method that can be widely implemented.

Ileal Digestibility of Amino Acids - Current Methodology and Possible Approaches to Standardization. H.H. Stein, University of Illinois-Urbana-Champaign, Urbana, IL, USA.

Not all amino acids that are ingested are also absorbed and utilized by the body, but the quantities of absorbed amino acids may be estimated by determining the ileal digestibility of amino acids. However, ileal digestibility is usually not determined in humans, but the pig is recognized as an appropriate model for humans. Determining ileal digestibility of amino acids in diets or food ingredients fed to pigs involves the surgical installation of a cannula in the distal ileum of pigs, which allows for collection of digesta samples directly from the distal ileum. By subtracting the quantities of amino acids that are excreted in the intestinal fluids from the intake of amino acids, the apparent ileal digestibility is calculated. However, because the output of amino acids in the ileal fluids includes not only amino acids of dietary origin, but also amino acids of endogenous origin, a correction for the endogenous amino acids is needed and by doing so, values for the standardized ileal digestibility are calculated. These values are additive if different ingredients are mixed together and the digestibility of amino acids in the mixture may be calculated from the standardized ileal digestibility of amino acids in each ingredient.

Protein Quality of Heat-processed Feed Ingredients and Its Effects on Swine Nutrition. F. Almeida and H.H. Stein, University of Illinois-Urbana-Champaign, Urbana, IL, USA.

Plant proteins commonly used in swine diets are routinely heat-processed. The application of heat is unavoidable and necessary to deactivate antinutritional factors (e.g., trypsin inhibitors), as well as to aid in the desolventizing and drying processes. Heat-processing, however, must be carefully controlled because overheating may initiate Maillard reactions. During these reactions, which occur under proper moisture and temperature conditions, the amino group of an amino acid or protein reacts with the carbonyl group of a reducing sugar. Lysine is particularly susceptible to these reactions because of its exposed epsilon amino group. Amino acids participating in Maillard reactions may be destroyed or become biologically unavailable to pigs. Thus, the concentration and digestibility of amino acids in heat-damaged feed ingredients is reduced. Consequently, reduced protein utilization by pigs fed heat-damaged feed ingredients may result in decreased growth performance and increased N excretion, causing environmental concern. Therefore, rapid and reliable evaluation of the protein quality of heat-processed feed ingredients is necessary to minimize their detrimental effects and efficiently use them in swine feeding programs.

A Molecular Look at Whey Protein Enriched Snack Foods. P.X. Qi, US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA.

Major class of proteins in milk, whey proteins contain a good amount of branched-chain amino acids (BCAAs), which are essential to stimulate protein synthesis in the body. Whey proteins are increasingly used in a variety of foods, from ice cream to healthy snacks including cheese curls and corn chips through extrusion texturization. Texturization uses mechanical shear, heat, and pressure to change the structures and thus the functional properties of food components, including proteins. Texturized whey protein (TWP) may then be used in a subsequent extrusion to obtain a snack food. To explore the complex relationship between processing conditions and functional and nutritional properties of the food products containing whey protein isolate (WPI), we studied the effect of extrusion temperature (eT) and moisture levels (eMC) on changes in the composition, molecular structure, and protein quality of the TWP. We showed that eT is a dominant factor in affecting the overall quality of the TWP as determined chemically by available free sulfhydryl and primary amines. Spectroscopic analyses based on circular dichroism and tryptophan fluorescence revealed considerable structural disruptions. Results from this work established that both eT and eMC can affect the structures and quality of TWP, and to limit losses and alterations, mild eT (50°C) and eMC (30-40%) are preferable.

Dietary Protein and Human Gastrointestinal Tract Health. K.A. Power, Guelph Food Research Centre, Agriculture and Agri-food Canada, Canada.

Dietary components, including dietary proteins, can modulate colon health components and impact human health and disease processes. In this study, the effects of purified flaxseed protein (FP) on colon health and disease (i.e. inflammatory bowel disease (IBD)) were determined in mice. C57Bl/6 mice were fed basal diet (BD; 20% casein) or BD supplemented with 20% FP for 3 weeks. During the last 5 days, half of the mice were administrated 2% dextran-sodium sulfate (DSS) in water to induce colitis. The effects of FP on colon health in healthy mice were assessed through histomorphometry and microbial activity. FP increased cecum weight and SCFA concentrations indicating increased microbial activity. Furthermore, FP modulated gut barrier integrity by reducing crypt length. The effects of FP on colonic inflammation were assessed in DSS-treated mice by disease activity index (DAI) scoring, and histological and molecular biomarkers of colitis. In colitic mice, FP aggravated disease severity as indicated by increased DAI and colon histological damage. Collectively, these results indicate that the type and source of dietary protein plays an important role in colon health and disease. Further studies are required to understand the mechanisms of FP adverse effects in the colon and the implications for human IBD.

Dietary Protein Consumption to Promote Human Health. W.W. Campbell and S.L. Gordon, Purdue University, West Lafayette, IN, USA.

Dietary protein is essential to support human health across the life span. Established perspectives exist that the dietary protein needs of old and elderly adults are not known with confidence and the currently established estimated average requirement (EAR) of 0.66 g/kg/d and recommended dietary allowance (RDA) of 0.8 g/kg/d may not be

accurate (too low) for this medically vulnerable population. Also, the scientific methods currently used to determine protein needs are not well-suited to quantifying metabolic, physiological, functional, and health-related parameters. Emerging research shows that old/elderly adults may benefit from consuming protein intakes moderately above the RDA to support good health, promote recovery from stress and illness, and retain physical function. For example, older adults who consume greater amounts of protein may have higher bone mass, muscle mass, and strength. Higher protein consumption by older adults with chronic conditions such as sarcopenia, osteoporosis, obesity, and diabetes is associated with more favorable clinical outcomes. However, new research using novel methodologies is needed to update and improve the scientific foundation for how much protein old and elderly people should consume to counter the catabolic effects of advancing age and morbidities and to promote health.

PCP 2: Bioactive Proteins and Peptides in Health and Disease I

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

A Novel Hypocholesterolemic Pentapeptide, Lactostatin, Inhibits Cholesterol Absorption via the Suppression of ABCA1 Gene Expression in Caco-2 Cells. S. Nagaoka, Department of Applied Life Science, Gifu University, Japan.

Lactostatin (Ile-Ile-Ala-Glu-Lys: IIAEK) is the first identified hypocholesterolemic pentapeptide derived from bovine milk beta-lactoglobulin by our group (1, 2). Lactostatin inhibits intestinal cholesterol absorption in Caco-2 cells and rats accompanying a hypocholesterolemia. However, the molecular mechanism of the inhibition of cholesterol absorption is unclear. We tried to clarify the molecular basis of the inhibition of cholesterol absorption by lactostatin in Caco-2 cells, a human intestinal model. To identify the target gene of lactostatin, we investigated the effects of lactostatin on intestinal cholesterol metabolism genes. Lactostain induced the suppression of ABCA1, MTP and CYP27A1 mRNA levels in Caco-2 cells. The protein level of ABCA1 and its gene promoter activity determined by luciferase assay are decreased by lactostatin treatment. Using the ABCA1 gene promoter assay, we tried to identify the target promoter region of ABCA1 gene mediated by lactostatin.

(1) Nagaoka, S., et al*., Biochem. Biophys. Res. Commun.* 281, 11-17 (2001)
(2) Morikawa, K., et al*., Biochem. Biophys. Res. Commun.* 352, 697-702 (2007)

Anti-inflammatory Effect of the Vasoprotective Di-peptides in Intestinal Mucosa System. Y. Kobayashi[1,2], Y. Mine[2], and T. Matsui[1], [1]Kyushu University, Fukuoka, Japan, [2]University of Guelph, Guelph, Ontario, Canada.

An anti-atherosclerotic di-peptide, Trp-His, has been shown to attenuate the development of vascular inflammation and regulate the intracellular Ca^{2+}-signaling pathways in part by its binding to L-type Ca^{2+} channels. Crohn's disease and ulcerative colitis are resulted from the chronic relapsing inflammation of the gastrointestinal tract. Our previous findings suggested that Trp-His can modulate chronic inflammation in intestinal mucosal system. The aim of this study was to demonstrate the anti-inflammatory effect of Trp-His. Trp-His (100 mg/kg) was orally administrated to a mouse model of acute colitis induced by dextran sulfate sodium (DSS). Trp-His significantly reduced the severity of clinical symptoms and also prevented the DSS-induced colon shortening. The local expression of pro-inflammatory cytokines (TNF-a and IL-6) in colonic tissues was strongly inhibited in mice treated with Trp-His. In addition, we elucidated that the underlying mechanism of reducing inflammatory responses by Trp-His in an intestinal epithelial cell, HT-29. Trp-His attenuated IL-8 secretion in TNF-a-stimulated HT-29. Its inhibitory effect of Trp-His on IL-8 secretion was completely abolished by a Ca^{2+} channel agonist, Bay K 8644. These results suggested the reduction of intracellular Ca^{2+} by Trp-His can be involved in the prevention of intestinal inflammation.

Novel Peptides from Lysozyme with Potential for Treatment of Inflammatory Diseases. H. Ibrahim, Kagoshima University, Faculty of Agriculture, Kagoshima, Japan.

The emergence of antibiotic-resistance and infection-associated inflammation are the foremost stressing clinical problems. This generates an urgent

need for new antimicrobial therapy with potential to control inflammation. Natural defense peptides have emerged as potential therapeutic agents as many exhibit immune modulatory activities. We could provide evidence that pepsin cleaves lysozyme (LZ) at specific loops to generate five antimicrobial peptide motifs. In this study, we explore the anti-inflammatory activities of these peptides. The peptides exhibit potent bactericidal action to various degrees against several bacterial strains and yeast C. albican and uniquely kill bacteria by dissipating bacterial respiration. All peptides, particularly helix-loop-helix and it N-terminal helix peptides, greatly suppressed production of the pro-inflammatory cytokines, interleukin-1ß (IL-1ß), interleukin 6 (IL-6) and tumor necrosis factor (TNF-a) from macrophages and human colon epithelial (HCT116) cells. This finding is the first to describe that LZ possesses multiple antimicrobial peptides while conferring potent anti-inflammatory activity and provides insight into new classes of food bioactive peptides that offer a fascinating opportunity for their potential use in the treatment of infectious and inflammatory diseases.

Suppression of Postprandial Hyperglycemia by an Amino Acid in *Allium* Plant. M. Akao, Y. Hirata, T. Kobayashi, and H. Kumagai, Nihon University, Fujisawa, Kanagawa, Japan.

S-Allyl-L-cysteine sulfoxide (ACSO) is a unique amino acid found in *Allium* plants such as garlic and Chinese chive. ACSO is odorless but has a function to enhance salty taste and umami taste. Our previous findings showed that ACSO was converted to active compounds after absorption from the small intestine, and exerted the inhibitory activity against platelet aggregation and the preventive activity against hepatic injury. However, ACSO itself may have some other physiological functions. Therefore, our study was conducted to focus on the suppressive effect of ACSO against postprandial hyperglycemia.

ACSO was orally administered to the rats for 7 consecutive days, and starch was given to the rats together with ACSO on the 7th day. Then, the blood was drawn from the tail vein, and the levels of glucose and insulin were measured. As a result, the postprandial elevation of blood glucose level was significantly suppressed by the oral administration of ACSO. In addition, the oral administration of ACSO lowered the secretion of insulin that was triggered by the increase in blood glucose level. Its mechanism of action will be discussed.

Induction of Oral Tolerance by Deamidated Wheat Gliadin. H. Kumagai[1], R. Abe[1], M. Akao[1], and H. Kumagai[2], [1]Nihon University, Fujisawa-shi, Kanagawa-ken, Japan, [2]Kyoritsu Women's University, Chiyoda-ku, Tokyo, Japan.

Wheat gliadin, an ethanol-soluble protein, is the major allergen of wheat-dependent exercise-induced anaphylaxis. As the IgE-binding epitopes of gliadin are tandem sequence repeats having glutamine residues, deamidation is effective to reduce wheat allergenicity.

Our previous findings showed that deamidation of wheat gliadin increased the solubility in water and reduced its allergenicity *in vivo*. If deamidated gliadin has a function to induce oral tolerance, it can be used for therapy of patients suffering from wheat allergy. Therefore, this study examined if deamidated gliadin has a function to induce oral tolerance.

First, mice were sensitized by intraperitoneal injection of wheat gliadin, and water (Control), untreated gliadin (UG) or deamidated gliadin (DG) was orally administered every other day for 4 weeks. Then, UG was orally administered a week after the final administration of each test sample.

When only water was administered for 4 weeks, oral administration of UG induced allergic reaction by enhancing the intestinal permeability, the expression of FceRI on mast cells, and the histamine and gliadin-specific IgE levels in plasma. However, after the oral administration of DG for 4 weeks, the allergic reactions were suppressed indicating that DG has a function to induce oral tolerance and would be a promising substance to cure wheat allergy.

Proteomic Approaches to Eggshell Membrane Bioactive Function. M. Hincke, C. Cordeiro, S. Smiley, and M. Rose-Martel, University of Ottawa, Ottawa, ON, Canada.

The avian egg is a complex reproductive structure which protects and nourishes normal embryonic development. Moreover, the egg's defenses resist bacterial invasion and maintain the sterile, unfertilized table egg as a highly nutritious foodstuff. It is necessary to understand its constituents that can serve as the basis for novel neutraceuticals for human health or development of enhanced eggs for human consumption. The eggshell, together with its cuticle and shell membranes, contains a proteinaceous matrix with potential pharmaceutical and therapeutic value. Recent proteomic studies of the eggshell protein constituents have identified candidate bioactive

molecules which are involved in the innate immunity of the egg due to their antimicrobial activity against invading pathogens. These include members of the BPI / LBP / PLUNC-like family, avian beta-defensins and other CAMPs that can be exploited for nutritional and neutraceutical applications.

Effects of Beta-conglycinin on Lipid Metabolism and Glucose Metabolism in Rats. K. Koba[1], E. Fukuda[1], S. Tamaru[1], D. Oikawa[2], and M. Sugano[3], [1]University of Nagasaki, Nagayo, Nagasaki, Japan, [2]Nagasaki University, Nagasaki, Japan, [3]Professor Emeritus, Fukuoka, Japan.

Feeding of beta-conglycinin, one of the major components of soy protein, was reported to decrease body fat mass, and serum and liver triglyceride levels in rats. Dietary beta-conglycinin was also observed to increase serum adiponectin concentration. In the present study, therefore, we investigated whether feeding of beta-conglycinin could affect factors influencing glucose metabolism as well, such as insulin sensitivity. Male Sprague Dawley rats were fed the AIN-93G diets containing 20% protein; either casein (CAS), or CAS replaced with soy protein isolate (SOY) or beta-conglycinin (CON) at the proportion of 50% for 1 month. After 4-week feeding period, we conducted insulin tolerance test. Compared with CAS, SOY and more clearly CON decreased blood glucose level past 90 min after intraperitoneal insulin injection (0.75 IU/kg body weight). The results suggested that SOY, especially CON could increase insulin sensitivity in rats. However, CON feeding affected neither fasting glucose level nor serum insulin level after the feeding period. Consistent with previous observations, dietary CON, compared with CAS, decreased white adipose tissue weights and liver triglyceride level. Therefore, results in the present study suggested that dietary CON could affect glucose metabolism as well as lipid metabolism in rats.

Protein Hydrolysates from Brewers' Spent Grain Enhance the Antioxidant and Immunomodulatory Potential of Food Formulations. N.M. O'Brien[1], A.L. McCarthy[1], Y.C. O'Callaghan[1], A. Connolly[2], C.O. Piggott[2], and R.J. FitzGerald[2], [1]University College Cork, Cork, Ireland, [2]University of Limerick, Limerick, Ireland.

Bioactivity of foods fortified with brewers' spent grain (BSG) phenolic extracts or protein hydrolysates was assessed following a simulated static gastrointestinal in vitro digestion procedure. Snack-bar and chocolate-drink formulations and a commercial yogurt product were fortified with BSG phenolic extracts or BSG protein hydrolysates and then subjected to in vitro digestion. Cytotoxicity of the fortified food digestates was measured using the MTT assay. Non-toxic concentrations of 0.5 % (v/v) and 0.1 % (v/v) digestates were selected for further experiments in Caco-2 and Jurkat T cells, respectively. The digest from yogurt supplemented with a phenolic extract significantly ($P < 0.05$) protected against H_2O_2–induced DNA damage in Caco-2 cells as measured by the comet assay. Jurkat T cells were stimulated with concanavalin-A (conA) and incubated with digestates for 24 hr prior to determining production of a range of interleukins (IL-2, IL-4, IL-10) and interferon-γ (IFN-γ) by ELISA. Addition of protein hydrolysates significantly ($P < 0.05$) increased the IFN-γ reducing capacity of the snack-bar. Hydrolysate fortified yogurt digests reduced IL-2 production to a greater extent than unfortified yogurt ($P < 0.05$). In conclusion, select BSG extracts possess the ability to enhance the antioxidant and anti-inflammatory potential of food formulations.

Anti-amyloidogenicities of Catechol Lignans Converted from Sesamin and Sesaminol Under Subcritical Water Condition. S. Nakamura and S. Katayama, Shinshu University, Minamiminowamura, Nagano, Japan.

Sesamin is a major lignin in sesame oil, and its biological effects have been studied extensively. Sesaminol contained in the soluble fraction of defatted sesame seeds powder also shows many bioactivities such as anticarciogenic and antiatherogenic activities in addition to antioxidant effect. Recently, we found that biological activities of these lignans metabolites were drastically improved compared with those of native forms, in which degradation of methlylenedioxybezene to catechol was observed. Although metabolites carrying with catechol moieties can be synthesized chemically, complex steps will be required. Thus, we employed a promising procedure named subcritical water treatment to prepared catechol lignans from sesamin and sesaminol. Changes of anti-amyloidogenicities of catechol lignans will be discussed in the presentation. Anti-amyloidogenicities of catechol lignans were determined using human cystatin L68Q, human stefin A & B, apolipoprotein A-II, and $Aß_{1-42}$ as target protein/ peptides. Effects of oral administration of

catechol lignans in senescence-accelerated mouse prone 8 (SAMP8) were also assessed. As a consequence, we found that catechol lignans possess anti-amyloidogenicities including preventing effects of learning and memory decline of SAMP8.

PCP 3: Bioactive Proteins and Peptides in Health and Disease II

Chairs: N. Hettiarachchy, University of Arkansas, USA; and R. Aluko, University of Manitoba, Canada

Incorporation of 13C-labeled Glutathione into Rat Blood After Oral Ingestion. K. Sato[1], H. Yamada[1], E.Y. Park[1], W. Aoi[1], and S. Ono[2], [1]Kyoto Prefectural University, Shimogamo, Kyoto, Japan, [2]Toyama University, Gohuku, Toyama, Japan.

Our previous studies have demonstrated that oral ingestion of glutathione increases protein-bound form of glutathione in human plasma. These facts suggest that orally administered glutathione could be absorbed into blood circulation system. However, there is possibility that increased glutathione may be synthesized in human. The objective of the present study was to confirm absorption of food-derived glutathione into blood system using 13C-labeled glutathione.

13C-labeled glutathione was synthesized in liquid phase method. 13C-labeled glutathione was administered to rat by oral gavage at 50 mg/kg body weight. Portal and peripheral bloods were collected and fractionated to plasma and cell fractions. Plasma was mixed with 3 vol of ethanol. The glutathione in the supernatant and residues fractions were extracted with 5% TCA-2% 2-mercaptoethanol. The extracted glutathione was derivatized with AccQ reagent and subjected to LC-MS/MS analysis.

After ingestion of 13C-labeled glutathione, 13C-labeled glutathione increased in portal and peripheral blood both in ethanol-soluble and protein-bound fractions. In addition, increase of 13C-labeled glutathione was also observed in liver. These facts clearly indicate that orally ingested glutathione can be absorbed into blood system and delivered to liver.

The Possible Antihypertensive Properties of Oilseed Protein-derived Peptides. S.M. Mäkinen and A.M. Pihlanto, MTT Agrifood Research Finland, Biotechnology and Food Research, Jokioinen, Finland.

Hypertension is a significant public health problem worldwide. One important factor in the regulation of blood pressure is angiotensin I converting enzyme (ACE) and oxidative stress is a crucial causative factor for the hypertension. Bioactive peptides, as products of hydrolysis of food proteins, are the focus on current research and most studied peptides appear to be those that inhibit ACE *in vitro.*

Oilseed plants (*Brassicaceae* or *Cruciferae* family) are important in the global oilseed economy. We have studied the possibility to use flaxseed and rapeseed by-products as source of bioactive peptides. Protein hydrolysates showed inhibition against lipid peroxidation and ACE inhibition (IC50 0.004-0.16 mg/ml) and hydrolysis with alcalase or subtilisin produced most potent activity. Fermentation produced lower ACE inhibition than enzymatic hydrolysis. ACE inhibitory compounds in rapeseed hydrolysate were resistant to gastric digestion (Mäkinen et al. 2011; Pihlanto et al. 2012; Pihlanto and Mäkinen, 2013). Fraction of the ACE inhibitory peptides derived from rapeseed prevented blood pressure increase in a preliminary test on 2K1C rats.

Obtained results indicate that plant proteins are promising source for the production of peptides that can be utilized to develop foods for prevention of hypertension.

Integrated Bioinformatics Approach for the Discovery of Cryptic Peptides from Agri-food Resources. C.C. Udenigwe, Dalhousie University, Truro, NS, Canada.

Bioinformatics is well-positioned to advance the discovery of bioactive peptides and in identifying sustainable protein resources. Bioactive peptides have gained interest for use in health promotion and several promising peptides have been identified by a classical approach that appears limiting in terms of sample scope and extensive processing requirements. Using web-based bioinformatics tools, the most abundant protein on earth, ribulose bisphosphate carboxylate (RuBisCO), was investigated for the occurrence frequency of cryptic peptides and possible cleavage of the peptides with commonly used food-grade proteases. The results indicated a strong potential of RuBisCO for use as a

sustainable precursor of peptides with antioxidative, angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory activities. Moreover, experimental analysis showed that the RuBisCO hydrolysates exhibited the bioactivities. Moreover, other potentially bioactive peptides were identified using an integrated approach, and a new dipeptide was discovered and found to exhibit strong antioxidative potential compared to physiological glutathione. These observations indicate that bioinformatics can enhance bioactive peptide discovery, and demonstrate the prospects of RuBisCO for use in developing functional ingredients against chronic diseases.

Waste Not Want Not: Marine Resources and Bioactive Peptides. M. Hayes[1], R. Aluko[2], C. Fitzgerald[1], D. Rai[1], and M. Hossain[1], [1]Food BioSciences Department, Teagasc Food Research Centre,, Ashtown, Dublin, Ireland, [2]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada.

Marine resources including macroalgae and fish processing co-product streams are a rich, underexploited reservoir of bioactive components. By-products from fish and shellfisheries processing represent a serious environmental and economic problem due to inadequate disposal and/or costs associated with disposal at landfill. By-products may be viewed as valuable sources of bioactive compounds including functional and technologically relevant peptides.Fish species like *Capros aper* (Boarfish), blue whiting and herring may also be viewed as a novel resource for new ingredients. Macroalgae can contain up to 25% protein, depending on the season of harvest and this protein is often rich in the essential amino acids. The red seaweed *Palmaria palmata* is the particular focus of our work. We describe how renin and PAF-AH inhibitory peptides were generated and isolated from this species and how the hydrolysates prevented hypertension in spontaneously hypertensive rats *in vivo*. In addition, seaweeds are a rich source of betaines and DMSP. This invited presentation discusses:

Bioactive peptides from marine processing by-products and utilsation of underutilised fish species for the generation of bioactive peptides. *Pamaria palmata* as a source of renin and PAF-AH inhibitory peptides.

Seaweeds as a source of betaine ingredients.

Rice Bran Derived Pentapeptide-induced Apoptosis in Human Breast Cancer Cell Models (MCF-7 and MDA-MB-231). R. Li[1], N. Hettiarachchy[1], and M. Mahadevan[2], [1]University of Arkansas, Fayetteville, AR, USA, [2]University of Arkansas for Medical Sciences, Little Rock, AR, USA.

A pentapeptide derived from rice bran has shown anti-proliferative property on human breast cancer cells (MCF-7 and MDA-MB-231). The objective of this study was to investigate the features of pentapeptide-induced apoptosis in breast cancer cells (MCF-7 and MDA-MB-231). The MTS (phenazine methosulfate 3-[4, 5-dimethyl thiazole-2-yl)]-2, 5-diphenyl tetrazolium bromide) assay was used to evaluate the growth inhibition activities of pentapeptide in a time-dependent manner (24, 48, 72, and 96 hrs). The features of pentapeptide-induced apoptosis in cancerous breast cells were evaluated by morphological changes, DNA fragmentation, and caspases-3/7 activities. The levels of COX-2 and p53 were evaluated by ELISA kits. Pentapeptide has shown growth inhibition on MCF-7 and MDA-MB-231 cells and the morphological changes and DNA fragmentation were observed. Significant decreases in levels ($p < 0.05$) of COX-2 and increases in levels ($p < 0.05$) of p53 were detected after pentapeptide treatment. The results suggest that pentapeptide introduces apoptosis human breast cancer cells and can regulate the death signal by up-regulating the level of p53 in both cells lines and down-regulating the level of COX-2 in ER-positive MCF-7 cells.

Immunomodulatory Activities of Bioactive Peptides on Male Weanling Sprague-Dawley Rats. W. Yu, C. Field, and J. Wu, University of Alberta, Edmonton, Alberta, Canada.

Bioactive peptides have potential for improving health and prevention of chronic diseases. The objective of the study was to test the immunomodulatory properties of chicken protein hydrolysate. Immunomodulatory activities of the protein hydrolysates were first tested in vitro on human monocytic cell line U937 to measure interleukin-6 (IL-6) production after lipopolysaccharides treatment. In vivo study was performed on male weanling Sprague-Dawley rats (n=8). Supplemented at 5% (w:w, diet based), the protein hydrolysate could significantly enhanced the production of regulatory cytokine IL-10 production in poke weed mitogen (PWM) or LPS activated splenocytes as compared to the casein-supplemented control group. Cytokine analysis

found an decreasing trend on IL-2 production in splenocytes activated with PWM or ConA, suggesting a suppressed Th1 response. Increasing trends were observed for IL-1ß, TNF-a and IL-6 on splenocytes but not on peritoneal macrophages. Reduced NO production was found on peritoneal macrophages incubated with LPS for 24 and 48 hrs. Phenotype analysis revealed slight increases on T cell (CD3+), Th cell (CD3+CD4+) and Tc cell (CD3+CD8+). In contrast, a slight decrease of IL-2 receptor expressing Th cell (CD4+CD25+) was consistent with reduced IL-2 production from cytokine analysis.

Structure-function Properties of Rapeseed Protein-Derived Antihypertensive Peptides. R. Aluko[1], R. He[2], and X. Ju[2], [1]University of Manitoba, Winnipeg, Manitoba, Canada, [2]Nanjing University of Finance, Nanjing, China.

This work was aimed at isolation and functional characterization of antihypertensive peptides from various enzymatic digests of rapeseed proteins. Rapeseed protein isolate was digested with proteases such as flavourzyme, alcalase, proteinase K, thermolysin, and a combination of pepsin+pancreatin (PP) to produce protein hydrolysates. Antihypertensive peptides were isolated from the most potent hydrolysates, the alcalase and PP hydrolysates. Three peptides (LY, TF, and RALP) were isolated from the alcalase hydrolysate and shown to possess renin and angiotensin converting enzyme (ACE) inhibitions. LY (IC_{50}, 0.11 mM) had the most potent ($p < 0.05$) activity against ACE while RALP (IC_{50}, 0.97 mM) was the most potent ($p < 0.05$) against renin. A novel peptide, GHS was purified from the PP hydrolysate and shown to also possess dual inhibition of ACE (IC_{50}, 1.74 mM) and renin (IC_{50}, 1.09 mM) activities. At a dose of 30 mg/kg body weight dose, the following maximum SBP (mmHg) decreases were obtained after administration to spontaneously hypertensive rats: GHS (-17), LY (-26), RALP (-16) and TF (-12). The results showed that the presence of hydrophobic amino acids enhanced antihypertensive activities of peptides.

PCP 4: Value Added Co-Products in Oil Processing

Chairs: N. Shah, Solae LLC, USA; K. Campbell, Solae LLC, USA; and R. Green, POS Bio-Sciences, Canada

New and Potential Protein Ingredients: Review and Examples. N. Lindeboom, POS Bio-Sciences, Saskatoon, SK, Canada.

Research into novel sources of protein can lead to producing a wide array of functional ingredients. The commercialization of primarily plant-based protein ingredients from pea to camelina and algal to canola offers formulation possibilities that may not have been available in the past. However, the protein purity, quality and cost depend on the production process used. As such, the processes and cost of established protein sources such as soy and whey will be compared to other new proteins. The presentation will provide a review of some new protein materials that are entering the market as well as an overview of some of the current research into developing these ingredients and possible unique properties they offer.

Technoeconomic and Life Cycle Analysis of Commercial Production of High-value Canola Protein Fractions and Coproducts using Aqueous Extraction Technology. E. Mupondwa, J. Wanasundara, and X. Li, Science and Technology Branch, Bioproducts and Bioprocesses, Saskatoon Research Centre, Agriculture and Agri-Food Canada, Government of Canada, Saskatoon, SK, Canada.

This paper presents a technoeconomic and life cycle analysis of an aqueous fractionation technology to improve utilization of canola protein fractions. The fractionation process is different from the classical notion of total protein isolates and concentrates. Proteins are fractionated based on molecular properties, thus providing products composed of individual storage proteins of canola seed. The scaling up study using canola seed feedstocks grown in the Canadian Prairies demonstrated the feasibility of developing the fractionation process into an industrial level process. Capital investment and operating cost for industrial process are estimated, along with complete profitability and investment criteria. In addition, the paper also provides a full life cycle analysis (LCA) of the aqueous extraction process with respect to sustainable use of materials and energy consumption, characterized in terms of key environmental impact factors including global warming, acidification, eutrophication, ozone

depletion, abiotic depletion, and human toxicity.

Conjugation of Whey Protein Isolate to Durian Seed Gum Enhances its Interfacial Emulsifying Activity. B. Tabatabaee Amid and H. Mirhosseini, University Putra Malaysia, Selangor, Kuala Lumpur, Malaysia.

Durian seed gum (DSG) is a heteropolysaccharide-protein complex from the agricultural biomass waste of Durian fruit. This hybrid polymer has an interfacial emulsifying activity. The present work was conducted to investigate the effect of purification and conjugation processes on functional properties of Durian Seed Gum (DSG) used for stabilization of Water in Oil in Water (W/O/W) multiple emulsion. Whey Protein Isolate (WPI) was conjugated to durian seed gum through the covalent linkage. In order to prepare the conjugated hybrid polymer, covalent linkage of whey protein isolate to durian seed gum was obtained by Maillard reaction at 60 °C and 80% (±1%) relative humidity (RH). In this study, W/O/W stabilized by the conjugated hybrid A showed the highest interfacial activity and lowest creaming layer among all prepared emulsions. This indicated that the partial conjugation of whey protein isolate to durian seed gum significantly improved its functional characteristics in W/O/W emulsion. The present study suggests that the partial conjugation of durian seed gum with whey protein isolate can promote its interfacial emulsifying activity in W/O/W emulsion.

Production and Properties of Rapeseed Albumin. F. Pudel and R.P. Tressel, PPM Pilot Pflanzenöltechnologie Magdeburg e.V., Magdeburg, Germany.

Rapeseed proteins - which are present at about 20 to 25 % of dry seed weight – possess high nutritional value as well as promising functional properties. In comparison to other oilseeds rapeseed contains two major fractions of storage proteins with completely different properties, the 2 S albumin napin with a molar weight of 12 - 17 kDa and the 12 S globulin cruciferin with a molar weight of about 300 kDa.
Particularly, napin could be very interesting for all applications, in which animal albumins should be replaced, e.g. in vegetarian foods.

The presentation will describe both a new technology to produce napin with a purity > 95 % and its functional properties, like solubility, emulsification, film, gel and foam formation properties and even more.

Overview of Pulse Proteins in Food Systems. M.C. Tulbek, C. Wang, and P. Unatrakarn, AGT Foods Research and Innovation Centre, Saskatoon, SK, Canada.

Pulse proteins provide nutritional benefits including protein fortification, low calorie formulation, allergen-free, gluten-free, and lactose-free solutions, as well as yield and technological improvement solutions in formulated foods. Pulse proteins which are fine powdered materials produced from the milling of dehulled pulses can be used as natural sources of plant proteins. Pulse proteins are food grade ingredients which are used to increase the protein content of foods with unique aroma and colour properties. Pulse protein products offer non-GMO solutions with a high-quality amino acid profile. Blending, water absorption, oil absorption, foaming and emulsification properties of pulse proteins make them excellent natural solutions for any food formulations. In this presentation the use of pea, lentil and faba bean proteins in food systems will be elaborated.

Encapsulation of *Bifidobacterium adolescentis* with Legume Proteins and its Survival Under Stimulated Gastric Conditions and During Storage Within Commercial Fruit Juices. J. Wang, D.R. Korber, N.H. low, and M.T. Nickerson, University of Saskatchewan, Saskatoon, SK, Canada.

Bifidobacterium adolescentis (ATCC 15703) was entrapped within capsules prepared using pea (PP), soy (SP), faba bean (FP) and lentil (LP) proteins mixed with alginate. Within synthetic gastric juice, B. adolescentis trapped within PP, SP, FP and LP with alginate capsules showed a 1.9, 3.3, 5.1, and 5.5 -fold reduction in cell numbers after 2 h. The release of encapsulated B. adolescentis within simulated intestinal fluid over 3 h indicated that almost all of the entrapped B. adolescentis were released after the first 10 min regardless of capsule formulation. The survival of B. adolescentis entrapped within PP capsules was subsequently investigated during a 6 week storage period in three commercial fruit juices (orange, pineapple, and white grape juice) at 4 and 22 °C. Free and encapsulated B. adolescentis cells could survive in the presence of pineapple and white grape juice with a 3.8 and 3.1 -fold reduction in cell number after six weeks storage at 4 °C, and with a 2.4 and 2.2 -fold reduction at 22 °C, but not orange juice.

Potential Use of Plant Proteins in the Development of Edible Biodegradable Films. M. Nickerson,

University of Saskatchewan, Saskatoon, SK, Canada.

Over the past decade, there has been an increased interest surrounding the use of biodegradable edible films by the food packaging industry as a way to reduce their environmental footprint. As such, researchers have been investigating the use of natural biopolymer-based materials (e.g., protein-, polysaccharide- and lipid-based) as an alternative to synthetic petroleum-based polymers. These biopolymer-based packages are considered to be more bio-friendly. Depending on the composition, films may display excellent barrier properties to moisture, gases and aromas; have the ability to carry and deliver various additives (e.g., antimicrobial agents and antioxidants) for extended product shelf-life or improved quality; or help improve a product's structural integrity and handling characteristics. This presentation will give an overview of edible biodegradable films prepared using a variety of plant proteins, with special emphasis given to proteins derived from canola and soy.

Rapid, Membrane-based Method to Separate *cis*-fatty Acid Salts into Individual Components. G. Abhinaba, C.J. Zeman, and N. Bowden, University of Iowa, Iowa City, IA USA.

We recently developed the first membrane-based method to separate a mixture of fatty acids into its individual components. The key to this method was the addition of an amine that formed a salt with the fatty acids. All of the fatty acids that we studied readily permeated the membranes at similar rates, but the salts formed by the reaction between the fatty acids and amines had different rates of permeation. By careful optimization of the amine used, an initial mixture of stearic, oleic, linoleic, and linolenic acids was separated into highly pure, individual components. For instance, a mixture of stearic, oleic, linoleic, and linolenic acids that was only 75% pure oleic acid was purified at high yield to give a component of 99% pure oleic acid. This method was used to differentiate stearic acid salts from cis-fatty acid salts, oleic acid salts from polyunsaturated salts, and linoleic acid salts from linolenic acid salts. The formation of the salts was reversible. Thus, after the fatty acid salts were separated by the membrane, the amines were rapidly separated from the fatty acids which allowed the amines to be recycled. The membrane was fabricated in one step from two starting materials.

PCP 5: Advances in Protein and Co-Products: Functionality for Food and Non-food Applications

Chairs: C.C. Udenigwe, Dalhousie University, Canada; J. Wu, University of Alberta, Canada; and M. Hojilla-Evangelista, USDA, ARS, NCAUR, USA

Counter-Current Carbon Dioxide Extraction of Soy Skim. F. Eller, M. Hojilla-Evangelista, and J. Teel, USDA, ARS, NCAUR, Peoria, IL, USA.

The use of carbon dioxide in a counter-current fractionation column was investigated as a means to remove residual fat from soy skim after enzyme-assisted aqueous extraction of soybeans. The stainless steel column was 1.2 meters long with an internal diameter of 1.75 cm and filled protruded stainless-steel packing. The soy skim feed, containing ca. 1% fat, was fed into the top of the column using an ISCO syringe pump. The carbon dioxide was pumped into the bottom of the column with a pair of ISCO syringe pumps. The extracted soy skim raffinate was drawn out the bottom of the column with a syringe pump and the carbon dioxide exited the top of the column through a back pressure regulator and into a glass collection vial. Factors such as solvent to feed ratio, flow rate, temperature and pressure were investigated for their effects. The fat content of the soy skim was determined as well as changes to the proteins in the soy skim.

New Co-products From Grain-based Fuel Ethanol Production and Their Drying Performance. K. Liu[1], R.R. Milczarek[2], and F.T. Barrows[3], [1]USDA, ARS, Aberdeen, ID, USA, [2]USDA, ARS, Albany, CA, USA, [3]USDA, ARS, Bozeman, MT, USA.

Fuel ethanol production in the U.S. and elsewhere is an important and growing industry. In the U.S, about 40% of annual corn production is now converted into fuel ethanol. During co-product recovery, condensed distillers solubles (CDS) has to be mixed with distillers wet grains before drying due to CDS's recalcitrance to drying. This results in distillers dried grains with solubles, a major co-product of dry-grind ethanol processing. Recently, at USDA-ARS we developed chemical, physical, and

physicochemical methods for fractionating CDS. The effort not only results in several new co-products with value-added uses but also addresses the dewatering problem of CDS and makes it possible to dry each component alone. When convectively dried at 60°C, all the new fractions showed faster drying rates than CDS, except for the glycerol-rich fraction. To further demonstrate the improved drying performance of the new fractions, we used a drum dryer to dry a protein-rich fraction and CDS (the control). Results show that while both materials could be dried to a range of endpoint moisture contents, the dried protein-rich fraction exhibited a broader range of water activity and lighter color than CDS and that the new fraction can be readily drum-dried into a shelf-stable, flaked product.

Recent Research Progress of New Soybean Protein Processing Technologies in China. X. Sui[1,2], Y. Li[1,2], Y. Zhang[1,2], B. Qi[1,2], and L. Jiang[1,2], [1]College of Food Science, Northeast Agricultural University, Harbin, China, [2]National Research Center of Soybean Engineering and Technology, Harbin, China.

Soybean is a main agriculture product in China and has been used to provide oil and protein (tofu) since ancient times. This review covers the nowadays new soybean protein producing technologies in China. In China, the soybean processing capacity is continuously increasing with an average annual growth rate of 30%. The main soybean processing industries are allocated in Northeast region, Bohai Sea region, Huanghai Sea region, Changjiang river region, Zhujiang river region, and southwest region. The soybeans are applied to produce protein, oil, functional ingredients, phospholipids, lubricant, plastic and etc. The new soybean protein processing technologies in this review covers: new extraction technology of soybean protein isolates, alcohol continuously leached soy protein concentrate technology, non-extrusion method to produce soybean protein, soybean phospholipid production technology, soybean lecithin production technology, and as well as introducing some of our self-invented equipment and commercial products. This review aims to provide e a declarative description of the current status of new soybean protein processing technologies in China.

Composition and Functional Properties of Protein Recovered from Pennycress (*Thlaspi arvense* L.) Press Cake. M. Hojilla-Evangelista, G.W. Selling, and R.L. Evangelista, NCAUR, USDA-ARS, Peoria, IL, USA.

Pennycress (*Thlaspi arvense* L.) seed oil is being considered as alternative feedstock for biodiesel production. If the pennycress-based biodiesel venture is successful, then the seed protein (more than 20% content) could become a major co-product of the process. This study compared two methods for extracting the press cake protein and determined the composition and functional properties of the protein products. Proteins in pennycress press cake were extracted by using the conventional alkali solubilization-acid precipitation (AP) method or saline-based (SE) procedure (0.1M NaCl at 50°C). We recovered twice as much freeze-dried product with SE but the AP extract had substantially higher protein content (90% versus 63% for SE). SDS-PAGE showed seven major bands that resolved at MW less than 45 kDa. Amino acid profiles for both protein extracts showed superior nutritional quality than soybean and rapeseed protein isolates. Protein extracts from both methods had greater than 90% soluble proteins at pH 2 and 10, but the SE extract was markedly more soluble at pH 4-8.5 than the AP isolate (76-90% versus 4-80% soluble protein). Pennycress press cake proteins also showed excellent emulsification and remarkable foaming abilities under acidic, neutral, and alkaline conditions.

Synthesis and Characterization of Carboxymethyl Flaxseed Gum (*Linum usitatissiumum L.*) and its Rheological Properties. J. Liu[1], J.H. Shen[2], Y.Y. Shim[2], P.G. Burnett[2], and M.J.T. Reaney[2], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

Flaxseed gum (FG) has rheological properties that resemble gum Arabic. However, low dissolution rate in cold water, dull color of solutions, and low storage stability limit its broad utilization. Chemical modification is a potential strategy to minimize these drawbacks. We studied carboxymethylation as a chemical modification process to improve FG properties. The effects of reaction conditions on the relative degree of substitution (DS) were determined. The highest DS of 0.862 was obtained at temperature of 70°C in the presence of NaOH (7.0 M), molar ratio of monochloroacetic acid to FG (anhydroxylose equivalent) 10:1, and reaction time of 3 h. Further, the structure and rheological properties of the resulting polymers were characterized. CMFG surface smoothness and crystallinity was improved compared with FG while

the glass transition temperature was decreased. The apparent viscosity of CMFG (0.2402 Pa·s, DS = 0.862) was lower than that of the native FG (11.83 Pa·s) at the same concentration of 3.0 % (w/v). Both the moduli (G' and G'') of CMFG decreased with increasing DS which is probably due to the suppressed entanglements of FG polysaccharide chains. Results of this research will provide the basic information for future applications of flax gums.

PCP-P: Protein and Co-Products Poster Session

Chairs: B. Lamsal, Iowa State University, USA

1. **Flocculation of High Purity Straw Lignin.** G.J. Piazza*[1], J.H. Lora[2], and R.A. Garcia[1], [1]U.S. Department of Agriculture, ARS, ERRC, Wyndmoor, PA, USA, [2]GreenValue, Media, PA, USA.

Flocculation of non-sulfonated lignin, a coproduct of biomass conversion to ethanol, has been examined. In industrial process, acidification causes lignin insolubility which allows purified lignin to be isolated. Flocculation has been envisioned as a way to eliminate the use of strong acid in the industrial process. The flocculants Poly(diallyldimethylammonium chloride) (pDADMAC) and bovine blood (BB), a waste product of animal processing, caused lignin insolubility, while cationic polyacrylamide, chitosan, and soy protein PF 974 were ineffective. Turbidity determined optimal flocculant, but turbidity magnitude with BB was greater than expected. pDADMAC caused negative lignin Zeta potential to became positive, but BB-lignin Zeta potential was always negative. Insoluble lignin did not gravity sediment, and flocculant-lignin mixtures were centrifuged. Pellet and supernatant dry mass and corrected spectroscopic results were in good agreement for optimal pDADMAC and BB. Spectroscopy showed 87-92% loss of supernatant lignin. Nitrogen analysis showed BB concentrated in the pellet until the pellet became saturated with BB. Subtracting ash and BB mass from pellet and supernatant mass confirmed optimal BB. Low levels of alum caused increased lignin flocculation at lower levels of pDADMAC and BB, but alum did not affect optimal flocculant.

2. **Impact of Processing on Structure and Allergenic Properties of Brown Mustard (*Brassica Juncea*) 2S Allergen Bra J 1.** H.K. Marambe, T.C. McIntosh, B. Cheng, and J.P.D. Wanasundara, Agriculture and Agri-Food Canada, Saskatoon SK, Canada.

Mustard is a popular condiment and considered as a priority food allergen in Canada and Europe. In brown and oriental mustard (*Brassica juncea*), Bra j 1, a 2S napin is the primary allergenic protein identified. Present study evaluated the effect of different food processing practices, namely blending with acetic acid and NaCl, heating to 72°C, baking, autoclaving and Flavourzyme-assisted hydrolysis on structural and allergenic properties of brown mustard (BM) napin. The changes occurred in secondary and tertiary structures of BM napin were investigated with circular dichroism and fluorescence spectroscopies. Rabbit polyclonal antibodies were generated against the epitope peptide sequence of Bra j 1 (AE-Ab) because of the unavailability of mustard allergen patient sera. These antibodies were able to detect Bra j 1 molecules in the 2S napin protein fraction. The ability of BM napin to bind with AE-Ab was determined using non-competitive indirect enzyme linked immunosorbent assay (NCI-ELISA) and was used to quantify Bra j 1 levels in the seeds. The BM varieties grown in Canada contain 836-882mg napin/ g seeds with Bra j 1 level ranging from 36.00 ± 0.57 to 47.74 ± 2.56 mg/ g seeds. Processing regimes that involve heat and hydrolysis affect AE-Ab binding and protein structure alterations.

3. **Growth Inhibitory Effects of Gossypol and Related Compounds on Fungal Cotton Root Pathogens.** J.E. Mellon, M.K. Dowd*, S.B. Beltz, and G.G. Moore, Southern Regional Research Center, New Orleans, LA, USA.

Gossypol and four related compounds were tested for their inhibitory effects against fungal soil pathogens. At 100 µg ml^{-1}, gossypol, gossypolone and apogossypolone demonstrated strong inhibitory activity (= 90%) against *Pythium irregulare*, *P. ultimum* and *Fusarium oxysporum.* The two gossypolone derivatives also provided good inhibition against most of the *Rhizoctonia solani* isolates (= 80%), with gossypol providing somewhat reduce inhibition against these fungi (60-80%). When compared with gossypol, the methylated

gossypol derivatives were generally less inhibitory. Dose response effects of gossypol, gossypolone and apogossypolone were also determined against *P. irregulare*, *P. ultimum* and *R. solani*. Gossypol was a more effective inhibitor of *P. irregulare* (ED_{50} = 7 µg/ml) and *P. ultimum* (ED_{50} = 16 µg/ml) than was gossypolone or apogossypolone. All three compounds were less effective at inhibiting *R. solani* (ED_{50} values between 45 and 48 µg/ml). The compounds were found to be effective antifungal agents with activity against a wide variety of fungal soil pathogens. The methylated gossypol derivatives, often present in some cotton tissues at levels greater than gossypol (e.g., in root bark), did not appear to be as effective an antifungal agent as gossypol.

4. **Improvement of Adhesive Properties of Cottonseed Meal by Water Washing.** Z. He*, C. Chapital, H. Cheng, and M. Dowd, USDA, ARS, SRRC, New Orleans, LA, USA.

In search of a relatively low-cost but efficient adhesive, we comparatively tested the adhesive strength and water resistance of water washed cottonseed meal (WCM), and cottonseed protein isolate (CSPI) on maple and poplar veneers (1.59 mm thick) using hot-press pressure of 2.8 MPa and variable press temperatures of 80, 100, 110 and 130 °C . The adhesive bonding strength of the two adhesives on maple veneers increased continuously with the increase of press temperature from 80 to 130 °C, but the bonding strength of the adhesives on poplar veneers were nearly the same at 100 to 130 °C press temperature. Adhesive bond strength was also measured after water resistance tests using both veneers. For WCM specimens, as the press temperature increased, the bond strength improved. With a press temperature of 110 °C, both the dry and wet adhesive strength values for WCM (4.65 and 5.18 MPa, and 4.11 and 4.38 MPa, respectively, for maple and poplar) were comparable to those of CSPI (5.61 and 5.17 MPa, and 4.00 and 4.69 MPa, respectively, for maple and poplar). Thus, the lower-cost-preparation of WCM can be more competitive than CSPI as a candidate for substituting synthetic wood adhesives.

5. **Improving the Color of Glandless Cottonseed Protein Isolate.** M.K. Dowd* and S.M. Pelitire, Southern Regional Research Center, New Orleans, LA, USA.

Cottonseed protein products (meals, concentrates, and isolates) tend to be dark in color. Even isolates prepared from glandless seed with very low gossypol levels tend to darken as they are dried. For example, when water washed to remove salt cottonseed protein isolate produced a somewhat grayish/brown product with a Hunter *L*-value of 74.3±0.8. To try to reduce color development, a number of isolate wash treatments were tried prior to freeze-drying. Washing the isolate with 0.1% citric acid did nothing to reduce color formation (Hunter L-value = 73.8±0.3). Washing with 50% isopropanol (IPA) resulted in a lighter color (Hunter *L*-value = 81.3±2.1). Adding 0.1% citric acid to the 50% IPA further improved isolate color (Hunter *L*-value = 88.9±0.4). In contrast, adding 0.1% butylated hydroxyanisole (BHA) to the 50% IPA wash only slightly improved isolate color (*L*-value = 82.8±0.2) and adding both citric acid and BHA to the IPA wash did not improve the isolate color beyond than of the IPA wash with only 0.1% critic acid added (*L*-value = 87.4±0.1). The results suggest that color formation occurs as a result of oxidation of trace levels of additional phenolic compounds. Washing with aqueous isopropanol helps to remove these compounds, while adding citric acid reduces metal ions that promote and accelerate the oxidation process.

6. **Oil Body Proteins of *Camelina sativa* (L.) Crantz (False Flax).** S.P. Perera*[1,2], T.C. McIntosh[1], R.T. Tyler[2], and J.P.D. Wanasundara[1,2], [1]Agriculture and Agri-Food Canada, Research Centre, Saskatoon, SK, Canada, [2]Department of Food and Bioproducts Sciences, University of Saskatchewan, Saskatoon, SK, Canada.

Camelina sativa (L.) Crantz is an emerging industrial oilseed crop of family Brassicaceae. Camelina or False flax is grown for oil that is used in biofuel and lubricant production. The meal of camelina is high in protein (38-43%) similar to other crucifer oilseeds and a good feed protein source. The predominant proteins of camelina are seed storage proteins and oil body proteins (OBP), which could be useful in protein based bioproduct development. Very little information is available on camelina protein fraction or its individual proteins. This study was carried out to characterize OBP of camelina using various spectroscopic and microscopic techniques. Outcomes of this study will enable to develop recovery method for camelina OBP and develop applications.

7. **Gelation of Canola Protein Isolates.** J.H.J. Kim and M.T. Nickerson, University of Saskatchewan,

Saskatoon, SK, Canada.

Gelation of canola protein isolates (CPI) were examined as a function of concentration (5-9%), pH (3-9), NaCl (0.1, 0.5 M) and 2-mercaptoethanol (0.1, 1 %) during heating-cooling by oscillatory rheometry. The magnitude of the storage modulus (G') of the gel was found to increase with increasing concentration at pH 7.0, whereas the gelling temperature (T_{gel}) remained constant at ~88°C. In the case of pH, CPI did not gel a pH 3, however networks formed at pH 5 became stronger (higher G') as pH increased. T_{gel} at pH 5 and 7 were similar (~88°C), then declined at pH 9 (~82°C). Zeta potential measurements as a function of pH found CPI to be positively (+18.6 mV), neutral and negatively (-32 mV) charge at pH 3, ~5.6 and 9. Increases in NaCl from 0.1 to 0.5 M reduced the zeta potential at all pHs, but had little effect on T_{gel} or network strength. In the presence of 2-mercaptoethanol, networks became weaker indicating the importance of disulfide bridging within the network. Findings suggest that networks were strongest under more concentrated conditions, under alkaline conditions (away from its pI). Disulfide bridging, electrostatics and hydrogen bonding are all thought to have a role.

8. **Gel Forming Properties of Soy Protein Isolates.** J.H.J Kim* and M.T. Nickerson, University of Saskatchewan, Saskatoon, SK, Canada.

Gelation of soy protein isolates (SPI) were examined as a function of concentration (5-9%), pH (3-9), NaCl (0.1, 0.5 M) and 2-mercaptoethanol (0.1, 1%) during heating-cooling by oscillatory rheometry. The magnitude of the storage modulus (G') of the gel and gelling temperature (T_{gel}) was found to increase and decrease (~81→71°C), respectively with increasing concentration at pH 7.0. In the case of pH, SPI did not gel at pH 9.0, and G' declined as pH increased from pH 3 to 7. T_{gel} at pH 3, 5 and 7 was observed at ~85.6, ~46 and ~81°C, respectively. Gels formed at pH 5 earlier due increased protein aggregation near its isoelectric point (pI). Zeta potential measurements as a function of pH found SPI to be positively (+ 35.4 mV), neutral and negatively (-51 mV) charge at pH 3, 5 and 9. Increases in NaCl from 0.1 M to 0.5 M reduced the zeta potential at all pHs, caused a shift in Tgel from ~81 to 67°C, and increased G'. No gels were formed in the presence of 2-mercaptoethanol. Findings suggest that protein-protein aggregation induced either by increasing concentration or at pHs near its pI, along with disulfide bridging is important in network formation.

9. **Mechanical, Optical, and Water Vapour Barrier Properties of Edible Films Prepared from Legume Protein Concentrates, and Its Potential use as a Carrier for Nisin.** J. Wang*, N.H. Khan, D.R. Korber, and M.T. Nickerson, University of Saskatchewan, Saskatoon, SK, Canada.

Six legume protein concentrates (from faba bean, pea, great northern bean, lupin, lentil, and soy) were used to form films through casting, in the presence of various levels of the plasticizer, glycerol (50, 75 and 100% w/w), and their mechanical and physical properties were assessed. Overall, faba-based films formed in the presence of 50% glycerol performed better than the others, with tensile strength/elongation of 9.99±0.84 MPa/22.83±8.85%, puncture strength/deformation of 10.09±0.36 N/11.89±0.74 mm, elastic modulus of 152.97±14.87 MPa, opacity of 224.4±8.8 A•nm, and water vapour permeability of 0.56±0.01 g•mm/m2•h•kPa. Subsequently, the antibacterial agent nisin was added to the film to test its effectiveness as a carrier. Film antibacterial activity was investigated using an agar disk diffusion method and six food spoilage/pathogenic microorganisms, including: Aeromonas hydrophila, Brochothrix thermosphacta, Listeria monocytogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens, and Staphylococcus aureus. In all cases, inhibitory zones were observed, suggesting that the film was an effective carrier for nisin.

10. **A Viable Approach for Biological Detoxification of Oil Seed Cakes and Their Utilization with Special Reference to Simultaneous Production of Protease Enzyme by Solid-state Fermentation Using *Aspergillus Niger*.** K. Bhardwaj* and R.K. Trivedi, Harcourt Butler Technological Institute, Kanpur, Uttar Pradesh, India.

Present study describes the complete degradation of phorbol esters by Aspergillus Niger strain during solid state fermentation (SSF) of deoiled Jatropha curcas seed cake. Phorbol esters were completely degraded in 15 days under the optimized SSF conditions viz. deoiled cake 5.0 g; moistened with 5.0 ml distilled water; inoculum 2 ml of overnight grown Aspergillus niger ; incubation at temperature 30 _C, pH 7.0 and RH 65%. SSF of deoiled cake seems a potentially viable approach towards the complete degradation of the toxic phorbol esters. This method simultaneously induces the production of Protease enzyme by *Aspergillus Niger.* The maximum Protease

activities obtained were 709.16 mg/ml in jatropha curcus oil seed cake. With increasing emphasis on cost reduction of industrial processes, commercial availability value addition to agro-industrial residues, non edible oil cakes could be ideal source of support matrix for various biotechnological processes. Several oil cakes, in particular non edible oil cakes over potential benefits when utilized as substrate for bioprocesses.These have been utilized for fermentative production of enzymes,antibiotics.

11. **Emulsifying Properties of Chia Seed *(Salvia hispanica* L.*)* Proteins.** L.M. Julio[1], M. Segura Campos[2], S.M. Nolasco[3], D. Betancur Ancona[2], and M.C. Tomás*[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – (Facultad de Ciencias Exactas (FCE) UNLP – CONICET), (1900) La Plata, Argentina, [2]Facultad de Ingeniería – Universidad Autónoma de Yucatán, Mérida, Yucatán, México, [3]Facultad de Ingeniería, Dto. de Ingeniería Química (TECSE), Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Olavarría, Pcia. de Buenos Aires, Argentina.

Chia seed is a good source of protein (19-27g/100 g seed) and the high nutritional quality of its proteins has attracted the attention of researchers. The aim of this work was to evaluate the emulsifying properties of chia protein fractions as a function of their chemical environment and treatment conditions (heat treatment, pH). Isolation and fractionation of different chia protein species (globulin, albumin, glutelin and prolamin) were obtained. Furthermore, O/W emulsions (25:75 wt/wt) with sunflower oil and different protein fraction concentrations (0.02-0.06% g/ml of aqueous phase) without or with denaturation (100°C, 15 min) at different pH (3-9) using an Ultraturrax homogenizer (20,000 rpm, 1 min) were obtained. Destabilization kinetics was determined through the evolution of Backscattering as a function of time. Emulsions showed destabilization by creaming, being albumins and globulins the fractions that contributed to a more stable emulsions. D[4,3] diameters were in the range of 30-50 µm. Emulsions with 0.04% proteins at pH 7 and 9 exhibited more stability against creaming while heat treatment did not influence the emulsifying properties of the different protein fractions studied.

12. **Structural Design of Fat Mimetics Using Gelatin-OSA Starch Coacervates.** B.C. Wu[1], B. Degner[2], and D.J. McClements[1], [1]University of Massachusetts, Amherst, Amherst, MA, USA, [2]ConAgra Foods, Omaha, NE, USA.

The creation of high quality reduced-fat food products is challenging because the removal of fat compromises quality attributes. The objective of this research was therefore to create hydrogel particles from gelatin and octenyl succinic anhydride (OSA) starch as fat mimetics. The hydrogel particles were formed based on the ability of cationic gelatin and anionic OSA-starch to phase separate through complex coacervation. Mixtures of type A gelatin (0.5 wt%) and OSA-starch (0 to 2.0 wt%) were dispersed in double distilled water at room temperature. Upon acidification to pH 5, the mixtures formed molecular complexes due to electrostatic attraction between gelatin and OSA-starch. The influence of polymer ratio on the formation of molecular complexes was determined by turbidity and micro-electrophoresis analysis. Static light scattering and optical microscopy analysis revealed that mono-dispersed, oval shaped particles were formed with a mean diameter (d_{43}) ranging from 5 to 15 µm with the maximum particle size being achieved at 0.4 wt% OSA-starch. These particles maybe useful as texture modifiers or to encapsulate flavor oils to improve the sensory quality of reduced fat products.

13. **Optimizing Ethanol Production, Oil Partitioning and DDGS Quality in Integrated Corn/Soybean Biorefineries.** J. Sekthon[1,2], L. Yao[1,2], L. Johnson[1,2], T. Wang[1,2], K. Rosentrater[2,3], and S. Jung*[1,2], [1]Iowa State University Department of Food Science and Human Nutrition, Ames, IA, USA, [2]Center for Crops Utilization Research, Ames, IA, USA, [3]Iowa State University Department of Agricultural and Biosystems Engineering, Ames, IA, USA.

Today, most fuel ethanol production is achieved by dry-grind processes by using corn grain. Thus, more than 10% of the 150 billion gal of annual motor fuels consumption can be met with corn-derived ethanol, but these plants are starting to close or operate under reduced capacity due to unprofitability. More efficient methods of production are desperately needed by the corn ethanol industry. The concept of integrated corn/soybean biorefinery could be a strategy to increase profitability of corn ethanol industry. In the current pathway for integrating corn/soybean biorefinery, two separate saccharification/ fermentation steps are implemented in order to apply conditions for optimal ethanol production from the corn and soybean fiber source, respectively. We investigated the potential of

combining these two saccharification/fermentation steps and identified the importance of cocktail of enzymes, saccharification and fermentation temperature and length, type of fermenting microorganisms (*Saccharomyces cerevisiae*, *Escherichia coli KO11*) on the three streams of the process: ethanol-enriched liquid fraction, thin stillage and enriched DDGS. This presentation will summarize the most important parameters impacting key factors influencing the economics of integrated corn/soybean biorefineries.

14. **Membrane Filtration for Enrichment of Phenolic Compounds in Olive Mill Wastewater.** R. Sheng*[1], S. Wang[1], I. Sedej[1], R. Milczarek[2], R. Avena-Bustillos[2], G. Takeoka[2], and G. Johnson[3], [1]University of California, Davis, Davis, CA, USA, [2]USDA - Agricultural Research Service, Albany, CA, USA, [3]New Logic Research Inc., Emeryville, CA, USA.

Olive mill wastewater (OMWW), generated during production of olive oil, is an untapped source of health-promoting phenols. Processors are aiming to separate OMWW into a high value, concentrated co-product stream and near-pure water that can be recycled back into the milling process. The objective of this study was to evaluate a membrane filtration technique for concentrating and isolating phenolic compounds from commercial 2-phase and 3-phase OMWW.

OMWW from three California mills (two 2-phase and one 3-phase) was collected and stored in a freezer for six months before treatment. OMWW was subjected to a sequential membrane filtration process using a novel vibratory system. Phenolic profile of the feed, permeate, and concentrate streams for each step in the process was analyzed by High Performance Liquid Chromatography (HPLC) with a Diode Array detector (DAD).

HPLC results confirmed that our sequential membrane filtration achieved the desired results of producing a phenol-rich co-product stream and a near-pure water stream that could be recycled into the milling process. Findings from this study will help olive oil processors add value to their co-product OMWW stream.

15. **Rapid, Membrane-based Method to Separate *cis*-fatty Acid Salts into Individual Components.** G. Abhinaba, C.J. Zeman, and N. Bowden*, University of Iowa, Iowa City, IA, USA.

We recently developed the first membrane-based method to separate a mixture of fatty acids into its individual components. The key to this method was the addition of an amine that formed a salt with the fatty acids. All of the fatty acids that we studied readily permeated the membranes at similar rates, but the salts formed by the reaction between the fatty acids and amines had different rates of permeation. By careful optimization of the amine used, an initial mixture of stearic, oleic, linoleic, and linolenic acids was separated into highly pure, individual components. For instance, a mixture of stearic, oleic, linoleic, and linolenic acids that was only 75% pure oleic acid was purified at high yield to give a component of 99% pure oleic acid. This method was used to differentiate stearic acid salts from cis-fatty acid salts, oleic acid salts from polyunsaturated salts, and linoleic acid salts from linolenic acid salts. The formation of the salts was reversible. Thus, after the fatty acid salts were separated by the membrane, the amines were rapidly separated from the fatty acids which allowed the amines to be recycled. The membrane was fabricated in one step from two starting materials.