2009 Annual Meeting Abstracts

Protein and Co-Products

MONDAY

MORNING

PCP 1: Protein Co-Products from Biofuel Productions

Chair(s): K. Liu, USDA ARS, USA; H. Wang, Iowa State University, USA; and J. Wanasundara, Agriculture & Agri-Food Canada, Canada

Corn Distillers Dried Grains with Solubles (DDGS): Opportunities and Challenges. Kurt A. Rosentrater, USDA, ARS, North Central Agricultural Research, Brookings, SD, USA

Corn-based ethanol in the U.S. has dramatically increased in recent years; so has the quantity of associated coproducts. Nonfermentable components are removed from the process as whole stillage, centrifuged to remove water - which is then evaporated to produce condensed distillers solubles (CDS), and then is recombined with the centrifuge solids and dried to produce distillers dried grains with solubles (DDGS). Each bushel of corn (56 lb) will result in nearly 2.9 gal of ethanol, 18 lb of CO2, and 18 lb of DDGS. DDGS are ~ 30% protein, 10% lipid, over 30% neutral detergent fiber, and up to 10% starch. Composition, however, can vary between plants and within a single plant over time. Distillers grains are primarily used as livestock feed, especially for beef and dairy, but also in swine and poultry as well. This helps to offset the use of corn for ethanol instead of feed or food. But there are many challenges associated with DDGS, including variability in nutrient content and quality; lack of an industry-wide quality grading system; inconsistent product identity and nomenclature; large quantities of energy required to remove water and the high cost of energy; moving DDGS to diverse and distant markets; potential mycotoxin contamination; and international marketing and export. Another challenge is poor flowability and material handling behavior.

Improving Co-products Composition by Dry Grind Ethanol Fermentation. Vivek Sharma[1], Robert Moreau[2], Vijay Singh[1], [1]Dept. of Agriculture and Biological Engineering, University of Illinois, Urbana, IL, USA, [2]Crop Conversion Science and Engineering, ERRC, ARS, USDA, Wyndmoor, PA, USA

Hominy feed is a low value coproduct of the corn dry milling process that accounts for nearly 35% of the starting corn quantity. The average composition of hominy feed on a dry basis is 56.9% starch, 25.2% neutral detergent fiber, 11.1% protein, and 5.3% fat. Starch in hominy feed can be fermented to ethanol thus increasing its levels of protein and fat. The increase in protein and fat percentages may increase the market competitiveness and price of hominy feed. Hydrolysis and fermentation were performed on nine hominy feed samples collected from three corn dry milling plants in the US. The original hominy feed samples and post fermentation solids were analyzed for starch, protein, fat and fiber content. Compared to the original hominy feed, the percentage increase in protein, fat and fiber in post fermentation solids of nine samples ranged from 10.4 to 21.3, 6.78 to 10.6 and 12.6 to 28.7% (dry basis), respectively. Ethanol yields varied from 271.7 to 380.2 L/metric ton for the nine hominy feed samples. These results are indicative that the value of hominy feed as an ingredient in animal diets can be increased with fermentation.

Using a new Modified Wet, Biorefinery System in an Ethanol Plant to Produce Novel Food Products. T. Lohrmann, D. Hammes, T. Adler, Quality Technology International, Inc., Elgin, IL USA

A majority of the ethanol plants today, or those currently under construction, are designed such that the entire corn kernel is ground prior to fermentation. The non-fermentable fraction remaining after fermentation is commonly dried to make it more shelf stable and sold into the livestock markets as a lower value feed product termed dried distiller grains with solubles. Recently we converted a 40 million gallon ethanol plant from a traditional dry grind system into a next generation, modified-wet fractionation bio-refinery capable of making food products such as high TDF corn bran and high purity corn germ. Since the biorefinery is a food grade facility and the fiber and germ are removed prior to fermentation, they make an excellent substrate for new food products. In contrast to the traditional wet milling system, this new modified-wet fractionation system is novel because sulfur dioxide is not added during the initial soak process.

Currently second generation, further-processed food products are in development which will bring even greater consumer benefits and value from this evolving industry.

Effect of Corn Breaking Method on Oil Distribution in the Thin Stillage of Dry-Grind Corn Ethanol Production. Hui Wang, Tong Wang, Lawrence Johnson, Iowa State University, Ames, Iowa, USA

One strategy to increase oil recovery from dry-grind corn ethanol production is through increasing oil distribution from solid fraction (wet distillerâ??s grains) to the liquid fraction (thin stillage) by physical treatment of the corn before fermentation. Several physical corn breaking methods were investigated in this study, including grinding, flaking, extrusion, and combination of them. A laboratory fermentation process and a bench-scale decanting procedure were successfully developed to stimulate the industry fermentation and decanting operation. It was found that oil distribution into thin stillage was positively correlated with the dry matter content of the thin stillage. The extractability of the oil in the slurry was also investigated. Flaking then high-shear-extrusion treatment released the highest amount of free oil. However, this treatment also produced the highest amount of fine particles, which led to the highest dry matter content in thin stillage. The combinations of low-shear-extrusion with grinding/flaking were also tested. All results indicate that it is difficult to increase oil distribution into liquid fraction without producing extensive fine particles.

Factors Affecting Fat Analysis of DDGS as Compared with Ground Corn. Keshun Liu, U.S. Dept. of Agriculture, Agricultural Research Services, Aberdeen, Idaho, USA

There are a few official methods for measuring oil/fat content in grains and feed. Yet guidelines on which method to be used for dried distiller grains with solubles (DDGS) are yet to be fully developed. In this study, a rapid determination of oil/fat utilizing high temperature solvent extraction (AOCS Approved Procedure Am 5-04) was used for measuring oil in ground corn and resulting DDGS. Factors, including sample type (ground corn and DDGS), sample variety (from ethanol plant 1, 2 and 3), sample particle size (original matrix, <25, and <35 U.S. standard mesh), solvent type (petroleum ether and hexane), extraction time (30 and 60 min), and drying time after extraction (30 and 60 min), were investigated by a complete factorial design. When measuring corn, only sample variety and extraction time had significant effect ($p < 0.05$) on oil values measured, but for DDGS, besides the two factors, sample particle size, solvent type and drying time also had significant effects. Among them, particle size affected most. On average, the measured oil content in DDGS ranged from 11.11% (original matrix), to 12.12% (< 25 mesh) and to 12.55% (< 35 mesh). In measuring oil in DDGS, particle size reduction, 60 min extraction and 60 min drying are recommended. Although petroleum ether gave a significantly higher value than hexane, the difference was relatively small.

Causes of Physical and Chemical Variability in Corn Distillers Dried Grains with Solubles (DDGS). Klein E. Ileleji, A.R.P. Kingsly, C.C. Clementson, K. Probst, Purdue University, West Lafayette, IN, USA

Variability in corn distillers dried grains with solubles (DDGS) is said to be one of the major reasons hindering the adoption of DDGS as a feed in livestock diets. Current production of DDGS in the U.S. is about 14 million tons from about 125 operating plants using corn with a total capacity of about 6.0 million gallons of ethanol. DDGS produced by a majority of the corn ethanol plants have high feed value due to its nutritive contents. However, the variability of DDGS product makes feed formulation by nutritionist difficult, especially when compared with its corn substitute. Past studies have shown that physical and chemical variability of DDGS is not just among plants but can vary between batch to batch from the same plant. Recent bench-scale and plant-scale research at Purdue University have identified the production process causes of DDGS variability and its effect on the chemical and physical properties of the product. This presentation and paper will bring the cumulative results of both the bench-scale and plant scale studies and discuss what can be done to minimize variability.

Co-Products of Biodiesel Manufacturing. M. Reaney, J. Shen, K. Ratanapariyanuch, C. Schock, R. Sani, University of Saskatchewan, Saskatoon, SK, Canada

A typical biodiesel plant using transesterification catalysis will produce at two products - biodiesel and glycerin. Both of these molecules are relatively low value commodities. However, it is possible to produce a wide range of value added products as diverse as health foods and industrial lubricants. Strategies for increasing the value of biodiesel

production facilities by recovering high value co-product streams including lithium grease, lubricity concentrates, phytosterols and conjugated linoleic acid will be discussed.

Separation of Protein and Corn Oil from Dry Milling Ethanol Plants. M. Dasari[1,2], M. Abdullah[1,2], [1]FEC Solutions, Des Moines, IA, USA, [2]Feed Energy Company, Des Moines, IA, USA

Byproducts from dry-mill ethanol production, conventionally sold as bulk livestock feed, are increasing at a significant rate and the supply may soon exceed the feed demand from the livestock industries. Specifically, high value corn fractions containing oil, limit the ability of livestock industries to increase the amounts of byproduct included in feed rations. With the lack of cost effective processes to extract value components from byproduct, an opportunity exists to develop technology to fill this emerging gap.The overall goal of this project is to demonstrate the feasibility of producing value-added co-products from the fermentation byproducts of dry-milling ethanol plants.

Pilot Process for Decolorizing/Deodorizing Commercial Corn Zein Products. D.J. Sessa, National Center for Agricultural Utilization Research, USDA, ARS, Peoria, Illinois, USA

Corn zein is the major protein component of ground corn, and co-products of the corn ethanol industry which includes distiller's dried grains and corn gluten meal. Zein products generated from those materials all possess some degree of yellow color and off-odor that deters their usage in food systems as well as in the medical, pharmaceutical and cosmetic industries. A pilot-scale process(patent pending) was developed to purify those products based on the protein and contaminants adsorption characteristics onto activated carbons (ACs) and zeolites (Zs), clay-based particles acting as molecular sieves. Statistical analyses of the binding characteristics of protein and contaminants for a series of ACs and Zs demonstrated that ACs from coconut hulls and Zs with a 5 Angstrom pore size proved ideal for adsorbing the least amount of protein relative to the adsorption of contaminants.These findings were used to select the media for packing the four columns of our pilot scale apparatus. The operating principles involve selective sequestration of the low molecular weight contaminants by continuous recycling of the column eluates. Sequential filtration proved to be an alternative to methodologies involving ultrafiltration/diafiltration on a tangential flow system that provided good recoveries of a purified zein product.

Tracking Lipid Levels during Ethanol Fermentation. C. Weller, E. Newgard, C. Leguizamon, Department of Biological Systems Engineering, University of Nebraska, USA

Producers using a dry-grind process to produce ethanol from corn and grain sorghum are actively searching for modifications that can be made to their process so as to extend their line of end products. Such modifications may result in valuable fiber, protein and lipid fractions. If such fractions become reality, a baseline level for each component in the various process unit operations and associated changes in the levels across the whole process need to be known for optimization. Observed lipid levels in unit operations of a bench-scale dry-grind ethanol production process using corn and grain sorghum will be presented. The observed lipids will be those reported to have health benefits when consumed. Speculation as to the reasons for changes in levels within or between unit operations will be described.

New Corn Degerming Processes and the Germ Quality. Hui Wang, Tong Wang, Lawrence Johnson, Iowa State University, Ames, IA, USA

A new degerming process was developed in this study. The germ yield, oil content in the germ, and the oil quality were investigated. It was found that this process effectively recovered the germ fraction from the corn and produced co-products with good purity.

AFTERNOON
PCP 2: Effect of Processing on Protein Functionality
Chair(s): S. Jung, Iowa State University, USA; and C. Onwulata, USDA, ERRC, USA

Effect of High Pressure Processing on Functional Properties of Food Proteins. M. de Lamballerie, H. Simonin, ENITIAA, BP 82225 44322 Nantes cedex 3 France

High pressure processing (200-800 MPa) is an alternative to traditional thermal food preservation methods, becoming current at the industrial scale, mostly for meat, seafood, fruit and vegetables. High pressure processing has limited effects on covalent bonds resulting in few protein modifications; however effects of high pressure treatment on weak bonds induce protein modifications. As food protein functional properties are linked with protein conformation, every property including water binding, emulsifying, foaming and gelling ability (consequently texture and rheology behaviour), enzymatic characteristics, colouring features and digestibility, may be modified by high pressure processing.This presentation will review main effects of high pressure processing on functional properties of food proteins, and will focus on our own results about:- several substrates: fish, meat, hen egg yolk, soy, and lupin proteins-high pressure at ambient temperature, and pressure shift freezing- texture, ultra structure, proteolytic activity (cathepsin and calpain), colour, emulsifying and gelling properties, and digestibility (related to allergenic properties).Finally the aim of this work is to improve the knowledge of effect of high pressure on proteins, to intend to favourably use main protein modifications under high pressure treatment.

Value Added Uses of Bitter Melon (*Momordica charantia*) Seed Protein (Emulsifying Property Enhancement). N. Hettiarachchy, R. Horax, P Chen, University of Arkansas, Fayetteville, AR, USA

Bitter melon is mainly used in Asia for food and medicinal purposes. The ripe seed is rich in protein. However, no information is available on its use as a functional protein.The objectives were to prepare bitter melon seed protein isolate (BMSPI), and investigate the functional properties before and after modification by glycosylation. BMSPI was prepared under optimum conditions (1.3M NaCl, pH 9.0). Glycosylation was conducted at varying relative humidities and temperatures using central composite design. Degree of glycosylation (DG), thermal and emulsifying properties were determined by fluorescamine assay, differential scanning calorimetry, and turbidimetry, respectively. The protein content of BMSPI was 90.2%. DG ranged from 39.3-52.5%, 61.7-70.9%, and 81.2-94.8% for treatments at 40, 50, and 60^{o}C, respectively (p-values <0.0001). No significant differences in the denaturation temperature were observed between unmodified BMSPI (113.2^{o}C) and glycosylated BMSPI (113.2-114.6^{o}C) with the exception of glycosylation at 60^{o}C (111.6-111.9^{o}C). Emulsifying activity increased from 0.35 to 0.80 for 80% DG or above, while emulsifying stability increased from 63 min to 72 min for above 70% DG. This glycosylated BMSPI with improved emulsifying properties can be used as an ingredient in foods where such properties are required.

A Mechanistic Study of Pea Protein Isolate - Gum Arabic Complex Formation. S. Liu, N. Low, M. Nickerson, Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Turbidity measurements were used to study the formation of soluble and insoluble complexes between pea protein isolate (PPI)-gum Arabic (GA) mixtures as a function of pH (6.0-1.5), salt concentration (NaCl, 0-50 mM) and protein-polysaccharide weight mixing ratio (1:4 to 50:1 w/w). For mixtures in the absence of salt and at a 1:1 mixing ratio, two structure-forming transitions were observed as a function of pH. The first event occurred at a pH of 4.2, with the second at 3.7, indicating the formation of soluble and insoluble complexes, respectively. Sodium chloride (≤7.5 mM) was found to have no effect on biopolymer interactions, but interfered with interactions at higher levels (>7.5 mM) due to substantial PPI aggregation. The pH at which maximum PPI-GA interactions occurred was 3.5, and was independent of NaCl concentrations. As PPI-GA ratios increased, structure-forming transitions shifted to higher pH. Complexation was greatest at a protein-polysaccharide weight-mixing ratio of 2:1. The nature of interactions involved during complex formation was subsequently studied in the presence of high levels of salt, urea, and as a function of temperature (6-60°C) to reveal mechanisms dominated by electrostatic forces, with secondary stabilization by hydrogen bonding and hydrophobic interactions.

Extraction and Characterization of Corn Germ Proteins. M.P. Hojilla-Evangelista, USDA ,ARS, NCAUR, Peoria, IL USA

Our study was conducted to develop methods to extract corn germ protein economically and characterize and identify potential applications of the recovered protein. Protein was extracted from both wet germ and finished (dried) germ

using 0.1M NaCl as solvent. The method involved homogenization, stirring, centrifugation, dialysis and freeze-drying. Factors evaluated were temperature (45, 55, or 65°C) and presence of reducing or denaturing agents. The recovered protein was analyzed for proximate composition and functional properties. Extraction was done at 45°C because no benefit was obtained by using higher temperatures. Addition of 2% SDS and 1% β-mercaptoethanol to the solvent nearly doubled protein yield; however, SDS-PAGE indicated protein denaturation. The recovered freeze-dried protein was least soluble (20%) at pH 2.0-4.0, but solubility increased gradually at higher pH. Wet germ protein extract was more soluble than finished germ protein at all pH values; however, the finished germ protein showed much better foaming and emulsifying properties.

Characterization of Protein Compositional Profiles and Structural Changes in Extruded WPI. P. Qi, C. Onwulata, USDA, ARS, ERRC, Wyndmoor, PA, USA

It was demonstrated that food products containing extruded whey protein isolate (WPI) possess beneficial nutritional properties and desirable texture. The effects of extrusion on the protein compositional profiles and molecular structures of WPI, however, remain poorly understood. In this work, we studied the effect of extrusion conditions including moisture and temperature on the protein compositional profiles by SDS-PAGE in the presence and absence of reducing agent, 1, 4-dithiothreitol (DTT). The molecular structural changes were investigated using circular dichroism (CD), FTIR and fluorescence spectroscopic techniques. The results showed that the extrusion moisture bears a clear positive effect on the water solubility of the proteins, but only negligible impact on the protein secondary structures. Increasing extrusion temperature, on the other hand, not only significantly reduces the water solubility but also considerably alters the protein composition and structures through the combination of shear and thermally- induced aggregation and denaturation. Quantitative analysis from gel electrophoresis suggested that among the two major protein components in WPI, β-lactoglobulin appears to undergo a greater conformational loss as a function of extrusion temperature compared to α-lactalbumin, presumably due to the formation of intermolecular disulfide bonds.

Functional Implications of Texturized Whey Proteins. C.I. Onwulata, P.X. Qi, A.E. Thomas, P.M. Tomasula, USDA,ARS, ERRC, Wyndmoor, PA, USA

Whey proteins are used in many food products to enhance nutrient content. Recent research findings are showing improved health benefits of whey proteins such as superior immune response. As spray dried whey protein powders, only limited amounts, less than 5 wt% can be used in formulated foods before unacceptable taste and textural thresholds are reached. To improve the physical compatibility of whey in formulations, and to boost the amounts that can be added up to 25 wt%, it was necessary to modify whey proteins functional groups and their conformation. We have used the extrusion processing conditions of moderate shear and temperatures (<100°C) to partially denature whey protein isolates. The process modification resulted in apparent loss (~15%) of secondary structures confirmed with Atomic Force Microscopy, and complete loss of globular structure at 75°C, and conversion to true random-coiled structure state at 100°C. High temperature extrusion formed distinct and uniform densely-packed structures which improved textural, functional, and nutritional properties. These textured whey protein isolates were used in formulated snack-type products.

TUESDAY

AFTERNOON

PCP 3: Bioactive Peptides in Human Health and Diseases

Chair(s): H. Kumagai, Nihon University, Japan; H. Ibrahim, Kagoshima University, Japan; and J. Wu, University of Alberta, Canada

Squeezing New Therapeutic Peptides Out of Egg Albumin. Hisham Ibrahim, Kagoshima University, Faculty of Agriculture, Kagoshima, Japan

Increasing attention is being focused on the science of bioactive peptides derived from food proteins. These peptides are inactive within the sequence of the protein molecule and can be liberated by gastrointestinal digestion or

proteolytic enzymes. This science involves the exploration of bio-activities of peptides from natural foods to formulate novel candidates for human health that may reduce the risk of disease. Egg albumen is a valuable source of bioactive proteins with diverse structural entities and many of them possess specific biological activities that represent potential ingredients of health-promotion. Thus, egg proteins offer tremendous opportunities for peptide drug discovery and hope for the treatment of emerging human diseases. In this work I will introduce our gastrointestinal simulation approach to uncover the potential bioactive peptides encrypted into egg albumin. Specifically, exploring novel bio-active peptides in egg albumin which heralding a fascinating opportunity for its potential candidacy as anti-infection and anti-oxidative bio-peptides for the treatment and risk reduction of emerging human diseases.

Occurrence of Pyroglutamyl Peptides in Wheat Gluten Hydrolysate and Its Beneficial Activity - Moderation of Hepatitis. K. Sato[1], M. Nagata[1], H. Sanada[2], Y. Egashira[2], S. Ono[3], Y. Suzuki[4], Y. Kido[1], P.Y. Park[1], K. Hashimoto[1], Y. Nakamura[1], [1]Graduate School of Life and Environment Sciences, Kyoto Prefectural University, Kyoto, Japan, [2]Faculty of Horticulture, Chiba University, Chiba, Japan, [3]Faculty of Engineering, Toyama University, Toyama, Japan, [4]Nisshin Pharma Inc., Tokyo, Japan

Gluten is formed from gliadin and glutenin during bread making and responsible for firmness of dough. Wheat gluten hydrolysate (WGH) has been considered as a stable glutamine source and prepared in an industrial scale. Beyond its nutritional value, there is an episode suggesting that ingestion of WGH could improve hepatitis of patients. The objective of the present study was to characterize peptides in WGH and confirm beneficial effect against hepatitis by animal models and identify the active compound in WHG. Up to half of glutamine residues in some WGH preparations were distributed in indigestible pyroglutamyl peptide fraction. Ingestion of WHG can improve galactosamin-induced acute hepatitis in rat model. Peptides in WHG were fractionated on the basis of amphoteric nature of sample peptide by the method of Hashimoto et al. The acidic peptide fractions showed significant moderation of the hepatitis. Peptides in the active fraction predominantly consisted of free pyroglutamic acid, pyroGlu-Leu, pyroGlu-Ile, pyroGlu-Gln, and pyroGlu-Gln-Gln. Among them, pyroGlu-Leu significantly suppressed the hepatitis, which can at least partially be responsible for the beneficial effects of WHG.

Reduction of Allergenicity and Improvement of Sensory Properties of Rice By Enzymatic Reaction During Cooking. H. Kumagai, Nihon University, 1866 Kameino, Fujisawa-shi 252-8510, Japan

Rice allergy is a serious problem especially for those where rice is consumed as a staple food. Different from patients with milk and egg allergies, children with rice allergy are unlikely to outgrow it, and are sometimes allergic to other cereals such as wheat, which makes their quality of life low during their lifetime. Although hypoallergenic rice products have been developed by the combination of several techniques such as enzymatic hydrolysis of allergens in degassed rice grains with surfactant or solubilization of allergens by salt solution under high pressure, their commercial availability is limited and their price is relatively high for daily use. We found that certain enzymes added to rice just before heating were active during cooking and could hydrolyze allergens. As some of the rice-improving substances used for large-scale cooking of rice contain enzymes, enzymatic treatment during cooking may not only reduce the allergenicity of rice but also improve the sensory properties of rice. Therefore, this study was aimed at examining the effect of enzymatic treatment on the allergenicity and sensory properties of cooked rice. The rice allergenicity was reduced and the sensory properties of cooked rice were somewhat improved by the addition of enzymes during cooking.

Anti-inflammatory Activity of Naturally Present Soy Peptides. E. de Mejia, V. Dia, University of Illinois, USA

Soybean contains several naturally present bioactive peptides. The objective of this study was to determine the *in vitro* anti-inflammatory activity of three naturally present soy peptides using RAW 264.7 macrophages. Isolation and purification was achieved by ion-exchange chromatography, ultrafiltration and size exclusion chromatography. The identity of the peptides was established by Western blot, HPLC, MALDI-TOF and LC/MS-MS. Fractions from both chromatographic techniques consistently showed three peptides with positive immunoreactivity against lunasin mouse monoclonal antibody. Treatment of RAW 264.7 macrophages with 100 µM of pure lunasin decreased the production of NO (92.6 ± 0.8%) and PGE2 (10.1 ± 4.5%), and the expression of iNOS (27.8 ± 2.1%) and COX-2 (41.4 ± 16.7%). Other two peptides, 8 and 14 kDa, had differential inhibition of COX-2/PGE2 and iNOS/ NO pathways. Possible

mechanisms of action will be discussed. This newly discovered property of soy peptides might contribute to the suppression of inflammation *in vivo*.

Regulation of Vascular Contraction by Peptides. Toshiro Matsui, Kyushu University, Fukuoka, Japan

Peptides, which are condensed amino-acids, have been well-documented as physiologically functional compounds in nature. To date, many functional food products with a health claim can be available in Japan. One of the successful products is an antihypertensive food including angiotensin I-converting enzyme (ACE) inhibitory peptides. However, their underlying mechanism of antihypertensive peptides still remains unclear, since some peptides did not show any inhibition effect on circulating ACE activity in human. We have thus investigated their physiological potentials on local blood pressure systems. We mainly focused on the vascular response of small di- and tri-peptides in rat thoracic aorta or cells, because they are favorably absorbed through intestinal peptide transporter, PepT1. In a series of our studies, we have clarified some physiological functions such as intact absorption into human circulatory blood systems and endothelium-independent vasorelaxant action via blocking of L-type voltage-gated Ca2+ channel. Among active tri-peptides, His positioned at the N-terminal as well as aromatic residues at the C-terminal was found to be essential for exerting the effect. These findings suggest that vasoactive small peptides may have an ability to improve vessel-related diseases including hypertension, diabetes or sclerosis.

Structure and Activity Relationship Study of Bioactive Peptides and Its Application in Generation of Novel Peptides. J. Wu, K. Majumader, University of Alberta, Edmonton, AB, Canada

Many kinds of bioactive peptides from various food proteins have been reported which have greatly advanced our understanding the structure and activity relationships of bioactive peptides. Through Quantitative Structure and Activity Relationship (QSAR) study, we have established the structural requirements of ACE inhibitory peptides; we have also preliminarily demonstrated that QSAR study could further facilitate the course of the discovery of new peptides with enhanced activity. However, the potential of QSAR models in revealing the most potent peptides has not yet been fully explored. We hypothesize that the most potent ACE inhibitory peptides can be produced through an integrated in silico digestion and QSAR study. In combination with computational technology, the potent peptide sequences in the protein structure have specifically released and their presences in the hydrolysate have been characterized by using LC-MS. The resulting scientific rationale could be applied to a wide range of bioactive peptide studies, and represents a quantum leap in the ability to discover new health-promoting bioactive peptides from food proteins.

Identification of a Cholesterol-lowering Protein Component in Soy. C. Schasteen, C. Jankovich, J. Wu, B. Pierce, B. Tulk, M. Mekel, D. Butteiger, E. Krul, Solae, Saint Louis, MO, USA

One possible mechanism to lower circulating cholesterol, a key benefit of soy in the diet, is to bind bile acids (BA) in the digestive tract. Cholesterol is the precursor of bile acids which are used in digestion to emulsify fat and aid in lipid uptake. BAs are effectively reabsorbed back into the body, however, an accepted mechanism to lower cholesterol is to reduce the BA reabsoprtion (increase BA excretion). BA excretion results in a decrease in serum cholesterol levels as the liver upregulates its uptake of plasma cholesterol for BA synthesis. Solae has an in vitro BA binding assay (BABA) that quickly measures soy's bile acid binding capacity and has shown that a Solae soy protein isolate, SPI, effectively binds BAs. Solae SPI has also been shown to lower circulating cholesterol in the male Syrian hamster, an accepted animal model of human lipid metabolism. BABA has been used to isolate the active component in soy and determine a molecular mechanism for soy's ability to reduce cholesterol. Solae SPI binds bile acids in our *in vitro* assay and this agrees with the increase in fecal BAs seen in hamsters suggesting that one of the mechanisms by which soy protein lowers cholesterol is via bile acid binding. We are currently fractionating Solae SPI to determine the molecular BA binding motifs in soy protein responsible for its inherent cholesterol lowering activity. Human clinical studies are being implemented to validate the results seen to date with *in vitro* BABA and *in vivo* animal models.

Multifunctional Peptides from Flaxseed Proteins: Antioxidant Properties and Suppression of Lipopolysaccharide-Induced Nitric Oxide Production in Macrophages. C.C. Udenigwe[1], W.-C. Hou[2], R.E. Aluko[1,3], [1]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada,

[2]Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan, [3]The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada

This study explores the production of peptides with potential human health benefits from flaxseed proteins (FP). A protein isolate was produced from defatted flaxseed meal followed by hydrolysis with pepsin, alcalase, ficin, trypsin, pancreatin, thermolysin and papain under optimum conditions. The protein hydrolysates were processed by ultrafiltration and ion-exchange chromatography to isolate low molecular weight and cationic peptide fractions. These peptides showed antioxidant properties in scavenging 2,2-diphenyl-1-picrylhydrazyl, superoxide anion, hydroxyl radicals and nitric oxide (NO), and also inhibited the activity of semicarbazide-sensitive amine oxidase. These results show that the peptides possess the potential for treatment of human diseases arising from oxidative stress. Antioxidant activities of these peptides were found to be dependent on the proteolytic treatments as well as the size of the peptides. The <1 kDa peptides from pepsin, ficin and papain FP hydrolysates also inhibited lipopolysaccharide-induced NO productions in RAW 264.7 macrophages; thus, these peptides may act as anti-inflammatory agents. These bioactive properties could encourage increased value-added utilization of flaxseed meal proteins for the formulation of therapeutic products.

Effects of Dietary Soy Protein on the Body Fat-reducing Potential of Conjugated Linoleic Acid in Rats.
Kazunori Koba[1], Asuka Akahoshi[2], Kazunari Tanaka[1], Michihiro Sugano[3], [1]University of Nagasaki, Siebold, Nagayo, Nagasaki, Japan, [2]Prefectural University of Kumamoto, Kumamoto, Japan, [3]Professor Emeritus, Kyushu University, Fukuoka, Japan

Conjugated linoleic acid (CLA) appears to reduce body fat in various animals. The magnitude of the effect differs among animal species, and humans are the least responsive. We previously suggested that the body fat-reducing potential of CLA could be increased by a combination with soy protein (SPI) instead of casein (CAS) in rats. Recently, β-conglycinin (CON), one of the main components of SPI has been shown to reduce serum triglyceride level more than SPI itself. Therefore, we examined how the combination of CON and CLA affects the lipid metabolism in rats. The rats were fed diets containing either 20% protein (CAS, SPI or CON) with 1% linoleic acid or CLA (9c,11t, 34%; 10t,12c, 36%). After 4 wk of feeding, dietary CON as compared with CAS and SPI lowered the body weight gain, which is due to decreased food consumption. Dietary SPI and more so CON decreased perirenal adipose tissue weights. This trend was more evident in combination with CLA irrespective of dietary protein. Serum triglyceride level decreased in the order of CAS, SPI and CON, and CLA tended to decrease the level further. The results suggested that body fat-reducing potential of CLA was more evident in combination with CON than with SPI. This effect could be partly due to decreased fatty acid synthesis and increased β-oxidation in the liver.

AM 3 / PCP 3.1: Alternative Sources in Aquafeeds
Chair(s): N. Vary, Canadian Food Inspection Agency, Canada; and K. Liu, USDA ARS, USA

The Beneficial Effect of Probiotics in Cultured Fish. T. Nakano, T. Yamaguchi, M. Sato, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan

In aquaculture, it is known that prevention of disease is more important than medical treatment. Part of disease prevention could come from daily diet. This concerns functional constituents, that is, food factors of feed other than essential constituents. Those functional feed ingredients have three categories; probiotics, prebiotics and biogenics. Probiotics (e.g. lactic acid bacteria) are defined as components of microbial cells or products from microbes that affect the host health. Prebiotics (e.g. oligosaccharides) are recognized as a nondigestible food ingredient that promotes the growth of beneficial intestinal microbes. Biogenics (e.g. polyphenolic compounds and vitamins) are defined as ingredients that modulate several functions of the body. Recently, some kinds of probiotics, in the wide sense, are often used under the assuming that they are equivalent to probiotics, prebiotics and biogenics are matched. Here, we will present some results of the effect of one of biogenics, polyphenolic procyanidins from grape seed, on the fish health. We found that procyanidins might improve antioxidant defense ability of fish. The use of probiotics in aquaculture should be regarded as a milder supplement therapy for fish and an environment-friendly method of aquaculture. We expect that ideal supplements for aquafeeds might be developed by the research of probiotics in the near future.

Nitrogen Utilization from Poultry Processing Co-products used in Diets for Florida Pompano. M. Riche, T. Pfeiffer, USDA, ARS, Fort Pierce, FL, USA

Feed represents the highest variable cost associated with fish production. High quality fishmeal (FM) continues to be the principal source of protein for fish, particularly carnivorous species that typically have dietary protein requirements of 40% or more. FM continues to be an expensive protein, with tight supplies when available, and little relief in sight. Replacement of FM with alternative protein sources will increase sustainability and profitability of the aquaculture industry. At the NOAA-USDA Alternative Feeds Initiative stakeholder's meeting in April 2008, poultry processing co-products were identified as top-tier candidates for evaluation as FM replacements in aquafeeds. However, one constraint to greater utilization of these co-products is the observed high variation in both quality and digestibility. It is well established processing conditions can affect amino acid availability in rendered products, and chemical composition and protein digestibility vary by product source. The goal of food fish production is the efficient conversion of dietary protein, measured as nitrogen (N), into salable fish flesh. This presentation reports on protein digestibility, amino acid availability, N excretion, and N retention in Florida pompano fed diets substituting five different poultry processing co-products as partial FM replacements.

Improvement of Nutritional Value of Lipid Sources by Monoacylglycerol Supplementation in Kuruma Prawn. Manabu Ishikawa, Shunsuke Koshio, Saichiro Yokoyama, Shinichi Teshima, Faculty of Fisheries, Kagoshima University, Kagoshima City, Kagoshima, Japan

This study presents effects of dietary lipid sources and monoacylglycerol (MG) supplementation on the growth and body lipid profiles for *M. japonicus* juveniles and larvae. Two feeding trials were conducted with 9 treatments (3x3), 3 lipid sources, triacylglycerols (TG), ethyl esters (EE), free fatty acids (FFA) and 3 supplements (control, Glycerol, MG). EE, FFA and MG were prepared from squid liver oil (SLO). In juvenile experiment, nine test diets were fed to prawns (body weight 0.4g) in triplicate 54-liter tanks. After 30 days feeding trial, body weight gain (BWG) and survival among the different treatments were statistically analyzed. Test prawn carried out to determine body fatty acid contents and the apparent digestibilities of dietary fatty acids. Results showed that there was a significant difference in BWG between the prawns fed the TG diets and those fed the EE diet. However, Supplementation of MG or glycerol trend to improve the growth of prawn in EE and FFA treatments. There were no significant differences in survival and total lipid content among the treatments. In larval experiment, similar trends were shown in the growth of the prawn. But glycerol supplementation was not effective for the improvement of larval growth.

WEDNESDAY

MORNING

PCP 4 / PHO 4: Protein Allergenicity and Regulatory Update on Phospholipids

Chair(s): J. Boye, Agriculture & Agri-Food Canada, Canada; J. Wanasundara, Agriculture & Agri-Food Canada, Canada; and W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands

Food Allergens and the Food Industry: Regulatory and Industry Perspectives Three Years After FALCPA. S. Taylor, University of Nebraska, Lincoln, NE, USA

The Food Allergen Labeling & Consumer Protection Act (FALCPA) in the U.S. and similar initiatives in other parts of the world have altered labeling practices and focused industry allergen control initiatives. In the U.S., FALCPA defined the commonly allergenic foods as milk, egg, fish, crustacean shellfish, peanuts, soybeans, tree nuts, and wheat. The common allergen lists differ in other countries. FALCPA mandates that the sources of ingredients derived from common allergenic foods shall be identied on the label in plain English language. This has been a major positive outcome for allergic consumers because it is now clear that casein is from milk and semolina is from wheat for example. However, FALCPA mandates labeling of allergenic sources on ingredients that may have quite low allergenic risks or that are present at rather low levels. Examples might include soy lecithin and fish gelatin. Exemptions from these labeling requirements are possible in the USA by petition but thus far no petitions have been granted. A much more workable petition process exists in the EU which has lead to the exemption of certain

ingredients from labeling. Advisory or precautionary labeling (e.g. "may contain") has proliferated even though none of the regulations or statutes mandate its use.

Lecithin Regulatory Update Including Residual Proteins. W. van Nieuwenhuyzen, Lecipro Consulting, Limmen, Netherlands

Food processors need additives for producing foods with specific functions and stable shelf life. In USA Lecithins have CFR and GRAS status and comply with FCC regulation. The EU permitted food grade additive Lecithin, including fractions and enzymatic hydrolyzed lecithin is classified as E322.Following recommendations of the Codex Alimentarius the Regulatory Authorities world wide require the labeling of foods (additives) derived from foods with allergenic substances for information of the consumer. Lecithin Manufacturing Associations (ILPS, ELMA) have investigated in collaboration with TNO Food Research Institute and other Institutes that most commercial soy lecithins may contain residual soy proteins. Results of Elisa tests will be presented. Alcohol soluble lecithin fractions may be free of residual protein.As a consequence, the sourced origins such as "soy", ?egg? or ?dairy? lecithin have to be declared on the food label. European food processors require ingredients for non-GMO crops, even if the GMO is officially accepted by regulation. Identity Preservation schemes for sourcing raw materials are in force.

Impact of Thermal Processing on the ELISA Detection of Food Allergens. T.-J. Fu, U.S. Food and Drug Administration, Summit-Argo, IL, USA

Commercial ELISA test kits are increasingly used by food manufacturers to validate allergen control measures. Many of these tests are designed to allow quantitative analyses where the presence of target proteins is detected by binding with specific antibodies. The concentration of the antigens is then determined from a standard curve generated with reference standards. Because quantitation is achieved via measurements of protein antigenicity, any changes in the antigenic property of the target protein may influence assay results. Thermal processing often leads to changes in the solubility and immunoreactivity of proteins. How thermal processing affects allergen quantitation by ELISA test kits remains to be determined. While many test kits employ antibodies that are raised against extracts of whole foods and are thus reactive to all protein components in these foods, other kits use antibodies raised against individual allergens and are therefore specific towards these proteins. Little is known about which tests are more suitable for detection of allergens in thermally treated food. This presentation discusses the impact of thermal processing on the performance of commercial ELISA kits for quantitation of egg, milk and peanut allergens. Factors such as the specificity of the antibody used, the thermal resistance of target proteins, and processing conditions used will be discussed.

Tree Nut Allergens. G.M. Sharma and S.K. Sathe, Florida State University, USA

Matrix Effects on Peanut Allergen Detection and Protein Extractability. J. Boye, N. Raymond, Agriculture and Agri-Food Canada, St-Hyacinthe, Quebec, Canada.

To assist in the detection of undeclared allergens in foods, reference materials with specified concentrations of targeted allergens are needed for the development and validation of allergen detection methods. In this study, protocols were developed for the preparation of peanut reference materials containing between 5 to 250 mg/kg of peanut using a peanut- and milk-free chocolate matrix. Specified amounts of peanut butter, defatted peanut flour and peanut protein isolate were added to pre-melted chocolate to give final concentrations in the desired range. The peanut extracts used were characterized using electrophoresis and RP-HPLC. Veratox and Tepnel Biosystems ELISA kits with and without extraction aids were used to measure the recoveries of the peanut proteins in phosphate buffer and the chocolate matrix. The results showed very low peanut recoveries in the chocolate matrices when no extracting aids were used. Recoveries improved with the use of extracting aids. Since sensitive peanut allergic patients can react to very low concentrations of peanut proteins, the research highlights the need for the use of extraction aids when detecting the presence of peanuts, especially, in chocolate matrices.

Identification and Characterization of Novel Soybean Allergens and the Creation of Hypoallergenic Lines. S.
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Gleddie , C. Gagnon , V. Poysa , E. Cober , Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada, [2]Agriculture and Agri-Food Canada, Harrow, Ontario, Canada

Food allergies affect an estimated 2-5% of the North American population. Soy allergy is an emerging concern because of its widespread use in the food and beverage industry, and it currently ranks high among foods provoking allergic reactions among North American and Asian consumers. Soybean meal also provokes significant adverse IgE-mediated allergic reactions when fed to weaning piglets. We are currently identifying and characterizing the major soy allergens by 2-D immunoblotting and mass spectrometry using sera from North American soy sensitive patients. We will describe the major soybean allergens including several novel allergens. A suprising finding of this study is that some patients who suffer allergic reactions to specific soybean proteins do not cross-react to very homologous proteins. Epitope mapping is proceeding to further define the regions or domains responsible in some allergens. We have also developed high throughput methods to screen germplasm for lines which lack allergens, an approach which we hope will lead to 'non-GMO' lines which are hypoallergenic for the food industry.

Impact of High Pressure and Thermal Processing on the Immunoreactivity of Sesame Protein Isolate. Allaoua Achouri[1], Joyce Irene Boye[1], Vincent Nail[2], Lamia L'Hocine[1], [1]Agriculture & Agri-Food Canada, St-Hyacinthe, Qc, Canada, [2]Université de Reims Champagne, Ardenne, Reims, France.

Sesame seeds are used for the preparation of a range of traditional dishes and as decoration and flavouring agents by the bakery industry. The increasing consumption of sesame-containing foods, and the fact that sesame may be present as a hidden allergen, is likely to make sesame allergy become even more common in future. Sesame seed has been added to the list of food allergens in European countries and Canada but not in the United States. Although several studies reported the existence of multiple allergens in sesame seeds, discrepancies persist in the literature due to factors such as the quality of extracts (purity), extraction conditions, food matrix and analytical techniques. Additionally, during food processing, proteins are subjected to various conditions, which lead to important conformational and structural changes that could be either beneficial or detrimental in terms of the immunoreactivity and/or allergenicity of the processed food. In the present study the extractability of sesame proteins under various conditions, their properties and the effects of high pressure (100-500 MPa) and thermal processing (boiling, dry roasting, microwave heating) on their immunoreactivity were investigated. Results obtained from these studies will be presented.

Recent Advances in the Development of Egg Allergy Immunotherapy. Y. Mine, M. Yang, University of Guelph, Guelph, Ontario, Canada

Increased awareness of food allergy and lack of efficient treatment regimens led to numerous investigations toward novel immunotherapeutic approaches. Recent epidemiological studies revealed that egg allergy prevails as one of the most common food hypersensitivities in industrialized countries. Egg allergens therefore constitute good models for the exploration of novel immunotherapies.Oral desensitization protocols have been assessed in patient cohorts with evidence of efficacy, but the approach remains associated with high risks of adverse effects and is unrealistic for highly-sensitive individuals. Safer and more efficient strategies are warranted. Identification and molecular characterization of egg components and their allergenic properties allowed the recent mapping of their T-cell and B-cell epitopes. These data have significantly facilitated exploration of elegant strategies, encompassing the use of non-IgE-reactive recombinant molecules and short T cell epitope-containing peptides. Promising results were recently obtained with use of murine models of egg allergy leading to elucidation of potential mechanisms involving regulatory T cells and key molecules such as TGF-b and FOXp3. Altogether, these investigations are expected to contribute to the successful development of clinically effective preparations for egg allergic patients.

Allergenic Proteins of Brown/Oriental and Yellow Mustard Seeds. J. Wanasundara, Y. Shim, Agriculture & Agri-Food Canada, Saskatoon, SK, Canada

Proteins that trigger allergenic reactions in human have been reported for yellow (Sinapis alba) and brown/oriental (Brassica juncea) mustard and caused the mustard allergenicity warning in Europe. In North America, mustard is a common condiment/spice particularly flour of yellow mustard is used in different foods. 2S proteins Sin a 1 of S. alba and Bra j 1E of B. juncea have been identified as molecules that are recognized by sera of mustard-sensitive patients.

Sin a 2, a 11S protein of yellow mustard (YM) has also been identified as immunoreactive. Two immuno-dominant regions in the large polypeptide chain have been identified for Sin a 1. The allergenic 2S mustad proteins have 8-Cys motif, multiple S-S bonds, high resistance to pepsin digestibility and high thermal stability similar to many allergenic 2S proteins. Using pAb raised against purified Sin a 1 and Bra j 1E, several mustard cultivars was assessed. The Sin a 1 content ranged from 0.75 to 2.29 mg/g meal and Bra j 1E content from 0.40 to 0.75 mg/g for yellow and brown/oriental mustards, respectively. Detected Sin a 1 level of YM flour was reduced due to temperature of heat treatment and the duration of the treatment with a maximum reduction of 80%. Heating of aqueous YM flour slurry >15 min at 100°C resulted in a same reduction of Sin a 1 level as heating dry flour at 125°C for 60 min.

Assessing and Managing Food Allergy Risks: An Industry Perspective. Rene W.R. Crevel, Unilever Safety & Environmental Assurance Centre, UK

Food allergy is now well recognized as a public health issue and in several regions of the world new regulations to protect allergic consumers reflect this. Even before this, many parts of the food industry, responding to the concerns of consumers, implemented systems to manage the risks arising from allergens. Much experience has now been gained with such systems and the general principles that govern good practice are reasonably well understood. However, it is not always clear whether the implementation of such systems has been accompanied by a concomittant decrease in the frequency of product recalls attributable to food allergens. This observation may indicate that allergen management systems are inadequate, but an alternative hypothesis is that they reflect a growing awareness of the issues and readiness to act. This presentation will briefly review the principles underlying allergen management, consider gaps in knowledge that limit the ability to assess the risk from allergens accurately and attempt to draw conclusions about the adequacy of current systems.

AFTERNOON

PCP 5: Industrial Utilization of Protein and Co-Products

Chair(s): G. Piazza, USDA, ARS, ERRC, USA; and R. Garcia, USDA, ARS, ERRC, USA

Electrospinning of Soy Protein Isolate Fibers for Controlled Release Applications. A.C. Vega Lugo, L -T. Lim, University of Guelph, Guelph, Ontario, Canada

In this study, an electrospinning method was developed to produce fibers with diameter ranging from 200 to 260 nm, which are much smaller than those produced by conventional wet spinning. Although denaturation of SPI at pH 12 and heating (60C for 2 h) was necessary to produce continuous fiber, this treatment alone was not adequate to initiate the electrospinning process. However, by incorporating a trace amount of poly(ethylene oxide) (PEO; ~0.8% w/w) into the protein solution, SPI fiber can be electrospun mainly due to the increased viscosity and decreased electrical conductivity. The SPI can be manipulated into various morphologies (fibers, beads/fibers, beads) by varying SPI:PEO ratio or using SPI from different commercial sources. Due to their large surface area and hydrophilicity, barrier and optical properties of the SPI fibers were highly relative humidity (RH) dependent. To exploit this behavior, a naturally occurring volatile antimicrobial compound, allyl isothiocyante (AITC) was incorporated into the protein solution and electrospun into fibers. Exposing the fibers to atmospheres with >75% RH effectively triggered the release of AITC. This effect may be leveraged for active food packaging applications to control the proliferation of spoilage microorganisms in food products with intermediate to high water activity.

The Performance Characteristics of Peptones made from Animal Byproducts. R.A. Garcia, G.J. Piazza, Fats, Oils and Animal Coproducts Research Unit, USDA-ARS, Wyndmoor, PA, USA

Fermentation is an increasingly important method for producing commodity chemicals. Such fermentations differ from laboratory or pharmaceutical fermentations by being larger in volume and more sensitive to costs. Operating costs for a fermentation process include the cost of growth medium ingredients, energy and labor, among others. Our previous work has shown that peptones capable of supporting the growth of various microorganisms can be produced from low cost animal proteins, including meat & bone meal, feather meal, and blood meal, through alkaline or enzymatic hydrolysis. In this work we explore how these experimental peptones compare to commercial peptones in terms of performance characteristics other than chemical make-up; these characteristics can impact operating cost by increasing

labor and energy use. It is shown that peptones with lower average molecular mass produce smaller increases in growth medium viscosity and foaming. Hydrolysates produced by alkaline hydrolysis produce a clearer, less colored growth medium compared to those produced by enzymatic hydrolysis, but they are more susceptible to precipitation and haze formation upon autoclaving, especially if $(NH_4)_2SO_4$ is present in the growth medium. All tested peptones are hygroscopic, which is problematic for storage, conveyance and dissolution. The influence of protein source and hydrolysis conditions on these factors is reported as well.

Effect of Functionalization on Protein Structure. N. Budhavaram, J. Barone, Virginia Tech, Blacksburg, VA, USA

Proteins or polypeptides are unique in nature in that they are chemically diverse because of the 20 different amino acids that could potentially comprise them. The diversity of functional groups exists for a reason specific to the biological system. The diversity of functional groups allows us to chemically modify the proteins to make peptide-based materials with non-natural properties. We have focused on a simple nucleophilic addition reaction known as the Michael addition that can add vinyl groups to amines and thiols under standard conditions. The results show that functionalization makes it possible to lower the glass transition temperature of proteins, order proteins, or make protein hydrogels.

Renewable Flocculants from Proteinaceous Agricultural Materials. G.J. Piazza, R.A. Garcia, USDA, ARS, ERRC, Wyndmoor, PA, USA

Blood, feather, and meat & bone meals are byproducts of abattoir operations. Use restrictions in recent years have caused excess supply problems, and research has been undertaken to find nonfood applications for these materials. Some proteins can act as flocculating agents. However, it is difficult to remove most of the protein from meals. Our investigations have revealed increased removal of proteinaceous materials from meals by partial hydrolysis using base treatment and enzymes. Hydrolysis reactions were sampled at different hydrolysis times. The partially hydrolyzed materials were tested for their ability to promote flocculation of finely divided clay particles. The molecular weight ranges of the hydrolysates were determined using gel filtration. We found that hydrolysates containing peptides above 5,000 daltons were effective flocculants, whereas those hydrolysates that consisted primarily of smaller peptide fragments were poor flocculating agents. At near neutral pH, concentrations of the hydrolysates at approximately 1.0 g/L were required for effective flocculation.

Protein-based Second Generation Bioplastics. J. Verbeek, L. van den Berg, University of Waikato, Hamilton, New Zealand

As with the production of biofuels from crops, there is major concern using these for bioplastics because of the perceived competition with food supply. Therefore, non-food resources are now being sought for the production of bioplastics. The aim of this study was to investigate the use of bloodmeal (BM) as a precursor for producing thermoplastic biopolymers. Bloodmeal, in combination with chemical additives, were compounded using a twin screw extruder and subsequently injection moulded into appropriate test pieces. Water in combination with other chemical additives were essential to reduce cross-linking and to sufficiently plasticise the polymer melt to allow protein chains to extend and interact. Successful processing resulted in a consolidated material with reduced cross-links and good homogeneity. It was found that at appropriate additive levels and correct processing conditions a material with a tensile strength of 10 MPa could be achieved. The corresponding Young's modulus and percentage elongation were 540 MPa and 13% respectively. This compared well with low density polyethylene, which has a tensile strength of 8 MPa and a Young's modulus of 200 MPa. However, the bioplastics was considerably less ductile when compared to a typical 400% elongation at break observed for polyethylene.

Canola Proteins in Bioplastics: Protein Characterization and Product Formulation. W.A.R. Manamperi, S.K.C. Chang, C.A. Ulven, S.W. Pryor, North Dakota State University, Fargo, ND, USA

Canola protein isolates were prepared via alkaline solubilization and acid precipitation for use in biobased plastics. Protein fractions with varying properties were isolated by precipitating at different pH values. Also specific proteins such as albumins, globulins, prolamins, and glutelins were isolated using the Osborn sequence separation procedure.

Functional and thermal properties of these isolates were evaluated to predict the functional behavior of these proteins in industrial applications such as plastics. Protein isolates were blended with polyesters to produce protein-based plastics using glycerol as a plasticizer and zinc sulfate as a cross linker. Mechanical and thermal properties of these protein-based plastics were studied.

Degradable Biopolymer Composites Made from Seed Proteins. S. Kim, USDA, ARS, NCAUR, Peoria, IL, USA

Zein is a prolamine of maize. Conventionally, 70-90% aqueous ethanol has been used to dissolve zein. Monitoring the variation of hydrodynamic radii of zein molecules in aqueous ethanol revealed that zein aggregates in the solvent and that the degree of aggregation depends on the composition of the solvent mixture. Zein has an amphiphilic characteristic whereby it forms micelle-like structure in its solution, and shows a molecular inversion as the composition of the solvent mixture changes. A wheat protein, gliadin, has similar characteristics as zein. The unique characteristics of these proteins are used for the production of degradable polymer composites from agricultural biopolymers. Micrometer-scale raw materials are coated with zein or gliadin and compressed to form a rigid material. Biopolymer composites produced with this technique showed promising mechanical properties. Incorporation of electrically conducting fillers such as carbon black and graphite into the biopolymer composite yielded highly conductive materials. The conductance of the final product was high enough to be used for commercial applications. The formation of nanoparticles from these proteins will also be discussed.

Advancement of Enzymatic Soy Hydrolysates in Adhesive Systems. J. Schmitz, Iowa State University, Ames, IA, USA

Varied protease and cellulase treatments to soybean flour result in soy flour hydrolysates with unique strength and water resistance properties in phenol formaldehyde and non-formaldehyde resin systems. Recent research has shown the degree of hydrolysis (DH) also plays a key role in utilization of soy flour in wood adhesive systems. Soy flour hydrolysates with 25% solids were produced with a DH as high as Δ26 and then blended with phenol formaldehyde resin for characterization in a wood product. Dry adhesive strength can be maintained in phenol formaldehyde resin blends with both low and high degree of hydrolysis up to 20% replacement, but with increased use of soy hydrolysate, water resistance of the adhesive bond decreased with increased degree of hydrolysis.

Soy Latex-like Adhesive for Wood Veneer Applications. G.Y. Qi, Kansas State University, Manhattan, KS, USA

Soybean protein has shown great potential for replacing petroleum-based polymers for adhesive application. Soybean protein modified with sodium bisulfite behaves like latex adhesives, with comparable adhesion strength to formaldehyde-based adhesives. The objective of this research was to investigate the compatibility of soy latex adhesive with six commercial wood veneer glues. Different levels of soy latex adhesive including 0%, 20%, 40%, 60%, 80% and 100% (total weight basis) were blended with wood veneer glues, and adhesion, rheological, thermal, infrared spectroscopy (FTIR) and morphological properties of the mixed adhesive were characterized. Dry adhesion strength of the soy latex were the same as all six wood veneer glues, and the dry adhesion of commercial wood veneer glues also maintained the same after blending with soy latex adhesive at various loading levels. Water resistance of wood veneer glues was improved by blending soy latex adhesive and wet strength was increased with the percentage of soy latex adhesive increasing. Viscosity of wood veneer glue were reduced significantly, and reached the lowest value at soy latex adhesive loading level from 40% to 60%. Thermal and FTIR studies showed that chemical reactions occurred between soy protein molecules and selected commercial wood veneer glues.

Protein and Co-Products Posters

Chair(s): P. Qi, USDA, ARS, USA

Favorable Conditions for Deamidation of Food Protein by Cation-Exchange Resin.
M. Ikeda, M. Akao, H. Sakurai, H. Kumagai, Nihon University, 1866 Kameino, Fujisawa-shi 252-8510, Japan

Deamidation is a useful tool to improve the functionality of food proteins. We have shown that cation-exchange resins of the carboxylate type effectively deamidates proteins without causing peptide-bond hydrolysis and that the deamidated soybean globulin has the novel functions of enhancing calcium absorption from the small intestine and preventing osteoporosis. In addition, the deamidated wheat gliadin shows increased foaming capacity, solubility in water and digestibility, and reduced allergenicity. However, the mechanism of deamidation by cation-exchange resins has not been clarified yet. This study was aimed at examining the favorable conditions for deamidation of food proteins by cation-exchange resins of the carboxylate type and analyzing the affinity of protein for the carboxyl groups by surface plasmon resonance-based biomolecular interaction analysis. The sodium concentration and pH were varied by using NaOH, HCl and NaCl. At strong alkaline pH with a high sodium concentration, the degree of deamidation of protein was high but peptide-bond hydrolysis was observed due to the alkaline hydrolysis. On the other hand, at a low sodium concentration and weakly alkaline pH, the interaction of protein with carboxyl groups became stronger, and high degree of deamidation occurred without causing peptide-bond hydrolysis.

Evaluation of Soy Peptides from R95-1705, N98-4445A, and S03-543CR Cultivars against Cancer Cell Proliferation.

S. Rayaprolu, N. Hettiarachchy, P. Chen, A. Kannan, University of Arkansas, Fayetteville, AR, USA

Soybean meal, a co-product after oil extraction from seeds, is rich in protein. Little work has been done towards value added uses of soybean meal. Our objective was to utilize this co-product, obtain protein isolates and peptides, and test for bioactivity against cancer, the second leading cause of death in the United States. Soybean cultivars with varying oleic acid contents were used for the study. Protein isolates were prepared and hydrolyzed using alcalase to generate peptide hydrolysates. After determining gastro-intestinal resistance, peptides were fractionated into definite molecular sizes of <5kDa, 5-10kDa, and 10-50kDa and tested against human colon (HCT-116) and liver (HepG2) cancer cells. Trypan blue dye exclusion assay and MTS cytotoxicity assay were performed to test *in vitro* cancer cell viability upon treatment with peptide fractions. The 10-50kDa peptide fraction from N98-4445A cultivar inhibits 70% of HepG2 and 73.64% of HCT-116 cell growth while <5kDa and 5-10kDa fractions from R95-1705 inhibit 52.7% of HCT-116 and 51% of HepG2 cells respectively. 5-10kDa fraction from S03-543CR inhibits 70% of HepG2 cell growth. Soybean peptide fractions can thus be a source of bioactivity against colon and liver cancer cell proliferation. This could translate soybean peptides to have potential nutraceutical and market value.

Kinetics of the Inhibition of Renin and Angiotensin I-Converting Enzyme by Flaxseed Protein Hydrolysate Fractions.

C.C. Udenigwe[1], W.-C. Hou[2], R.E. Aluko[1,3], [1]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada, [2]Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan, [3]The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada

Enzymatic flaxseed protein hydrolysate fractions were investigated for in vitro inhibition of angiotensin I-converting enzyme (ACE) and renin activities. Pepsin, ficin, trypsin, papain, thermolysin, pancreatin and alcalase were used to hydrolyze flaxseed proteins followed by fractionation using ultrafiltration to isolate low-molecular-weight peptides, and separation of the alcalase hydrolysate into cationic peptide fractions. Using N-(3-[2-furyl]acryloyl)-phenylalanylglycylglycine as substrate, the protein hydrolysates showed concentration-dependent ACE inhibition (IC50, 0.0275 to 0.151 mg/ml) with thermolysin hydrolysate and alcalase cationic peptide fraction I (FI) showing the most potent activity. The flaxseed peptide fractions also showed nil or moderate inhibitory activities against human recombinant renin (IC50, 1.22 to 2.81 mg/ml). Kinetics studies showed that the thermolysin hydrolysate and FI exhibited mixed-type pattern of ACE inhibition whereas cationic peptide fraction II inhibited renin in an uncompetitive fashion. These results show that the protein components of flaxseed meal possess peptide amino acid sequences that can be exploited as potential food sources of antihypertensive agents.

Biochemical Characterization of Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] Legumin.

G.M. Sharma[1], M. Venkatachalam[1], K.H. Roux[2], S.K. Sathe[1], [1]Department of Nutrition, Food & Exercise Sciences, Florida State University, Tallahassee, Florida, United States, [2]Department of Biological Science, Florida State University, Tallahassee, Florida, United States

Pecans are globally consumed as a snack food and used in many confectionary and baked products. Tree nuts are listed among the â??Big 8â? food groups which are responsible for more than 90% of allergic cases. Pecans are one of the tree nuts reported to cause allergies in sensitive individuals. IgE-mediated food allergies are caused by food proteins. Legumins, a class of cupin superfamily proteins, occur in several seeds, and are known allergens. We have cloned and expressed the pecan legumin for sequence analysis and future testing for allergenic properties.Pecans in late maturation stage were used for cDNA library construction. Primers were designed based on the conserved region of walnut legumin with other seed proteins, and used to amplify pecan legumin using a cDNA library as the template. Native legumin was isolated by Osborne fractionation and gel filtration chromatography.Two legumin clones were selected for sequencing. These clones, each 1518 bp long coding region, differed in 3 nucleotides (position 18, 906, and 1294) and 2 amino acids (position 302 and 432) indicative of isoforms. The protein sequence had 94% identity to walnut legumin. The N-terminal sequence of the native polypeptide (~25 kDa basic subunit) exhibited 88% identity with the recombinant counterpart.

Complex Coacervation of Pea Protein-Gum Arabic Mixtures.
D.R. Klassen, M.T. Nickerson, Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Turbidity measurements were used to study the formation of soluble and insoluble complexes involving gum Arabic (GA) with a mixed pea globulin system, and crude legumin (Lg) and vicilin (Vn) fractions, as a function of pH (6.00-1.50) at a 2:1 protein-polysaccharide weight-mixing ratio. Complexation typically follows two pH-dependent structure forming events, corresponding to the formation of soluble (pHc) and insoluble complexes. The formation of soluble complexes occurred at pH 3.88 for both the mixed globulin-GA and Lg-GA systems, whereas pHc occurred at a higher pH (4.05) for the Vn-GA system. In contrast, all biopolymer systems were found to form insoluble complexes at pH 3.64. Maximum protein-polysaccharide interactions for all systems occurred at pH 3.48, corresponding to conditions where complexes were electrostatically neutral (having a zeta potential of zero). Dissolution of complexes then occurred at a pH of 2.57. Crude Lg- and Vn fractions were subsequently purified using anionic exchange-fast protein liquid chromatography. Relative changes of the purified fractions to their secondary and tertiary structures after undergoing a pH-induced complexation with GA were studied by spectroflourometry and circular dichroism.

Protein Recovery in Enzyme-assisted Aqueous Extraction Processing of Soybeans.
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Enzyme-assisted aqueous extraction processing (EAEP) of soybeans produces a significant amount of protein-rich effluent (skim). In order to reduce effluent production, two-stage countercurrent EAEP was applied. Skims with different degrees of proteolysis were produced: skim 1, where 0.5% protease (wt/g extruded flakes) was used in both extractions; skim 2, where enzyme was used in the 2^{nd} extraction only; and skim 3, where no enzyme was used in either extraction. Protein extractions of 96, 90, and 65% were achieved when producing skims 1, 2 and 3, respectively. Protein recovery strategies using ultrafiltration (regenerated cellulose membrane) and upstream isoeletric precipitation were evaluated. Protein retentions of 93.0 and 98.8% and permeate fluxes of 1.1 and 0.3 kg/h.m^2 were achieved for skim 1 using 1 kDa and 500 Da membranes, respectively. Protein retentions of 99.4 and 98.9% and permeate fluxes up to 2.4 and 1.8 kg/h.m^2 were obtained in a double filtration (30 kDa/500 Da) of skims 2 and 3, respectively. Protein recoveries of 28.0, 62.0, and 85.7% were obtained with isoeletric precipitation of skims 1, 2, and 3, respectively. Ultrafiltrations (500 Da) of respective supernatants achieved protein retentions of 98.3, 96.8, and 92.5% and permeate fluxes of 0.9, 1.3, and 1.5 kg/h.m^2, respectively. High protein retentions were associated with low permeate fluxes.

Barley Protein Extractions and Their Functional Properties.
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Barley is the second most important cereal crop in Canada after wheat. Canadian barley is natural and the grain contains 8-16% proteins, which provide an interesting resource for extraction of Non Genetically Modified (Non-GMO) plant proteins. In this work, barley endosperm proteins were extracted by aqueous methods. Alcohol and alkaline methods resulted in 87% barley hordein and 81% gluten at the optimized conditions. The protein recovery was

90% and 65% for hordein and gluten, respectively. Functional properties of the barley protein isolates were evaluated in comparison to soy, whey, corn and wheat proteins. Barley proteins demonstrated unique emulsifying and foaming properties that may find interesting applications in food and cosmetic areas.

Isolation of Proteins from *Brassica juncea* Meal.

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Protein was extracted from ground defatted Brassica juncea meal using water with KOH and NaCl added to adjust the pH and salt concentration. The total yield of protein and the properties of the extracted protein were examined. Protein extraction efficiency was studied at different pH (7.6-10.4) and salt concentrations (3.4 x10-2-1.2M). Both pH and salt concentration of the extraction medium affected protein extraction efficiency. Optimum extraction efficiency was observed at pH 10 and a salt concentration of 1.0M. The extracted protein isolates from ground defatted meal were approximately 100 percent protein and had an isoelectric point of pH 6.4. SDS-PAGE and peptide sequencing showed that napin and cruciferin were the most prevalent proteins in extracted fractions at molecular weight approximately 14, 18-20, 20-22, 34 and 55 kDa. Amino acid composition of extracted protein was comparable to those of other researchers and the extracted protein had a high lysine content (5-6 g/100g of protein). Lysine availability and in-vitro digestibility of the concentrate were approximately 40 g/kg of protein and 75%, respectively.

Crystal and Molecular Structures of 6,6′-dimethoxygossypol.

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Gossypol is an unusual polyphenolic terpene found in many members of the Gossypeae plant tribe, including the cotton plant. The compound is studied because it is responsible for the antinutritive properties associated with the feeding of cottonseed and cottonseed meal, but is also of interest because it has potentially useful biological properties. Gossypol has unusually physical properties. Among these, it forms many different solid-state forms when crystallized under different conditions. 6-Methoxy-gossypol and 6,6′-dimethoxy-gossypol (DMG) are gossypol derivatives that are formed in fairly high concentrations in some *G. barbadense* cotton varieties. We have recently isolated these compounds to study their physical and biological properties. In this work, we report on the structures of four new DMG crystal forms. These structure include two non-solvated polymorphs and two different equimolar solvates formed with cyclohexanone and water. From this sampling, we find that in some crystallization conditions DMG forms solid-state structures similar to those formed by gossypol, but that in other conditions DMG and gossypol form different solid-state structures.

***In vitro* Bioactive Properties of Hempseed and Pea Seed Protein Hydrolysates.**

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Hempseed protein isolate and pea protein isolate were each separately hydrolyzed using food grade enzymes and followed by sequential membrane fractionation (1 kDa, 3 kDa, 5 kDa, & 10 kDa) of the hydrolysates. With the exception of the 10 kDa permeate which showed the least activities, there was no significant effect ($p>0.05$) of peptide size on measured activities. Decolorization of the hempseed protein isolate (to remove polyphenols) prior to enzyme hydrolysis did not have any significant effect ($p>0.05$) on the measured *in vitro* bioactive properties of the peptide fractions. The hempseed peptide fractions showed weak activities (at 1 mg/ml peptide concentration) against angiotensin converting enzyme (<13% inhibition) and DPPH (<11% scavenging). In contrast superoxide radical scavenging activity of the hempseed peptide fractions was moderate (up to 40%). Pea protein hydrolysate (PPH) displayed very strong, concentration dependent metal ion chelating activity with an effective concentration (EC50) value of 0.097 mg/ml but showed no DPPH scavenging activity. PPH also demonstrated strong hydrogen peroxide scavenging activity with an EC50 value of 0.716 mg/ml. We concluded that the hempseed and pea seed protein hydrolysates may be used as potential ingredients to formulate functional foods and nutraceuticals.