2010 Annual Meeting Abstracts

Protein and Co-Products

MONDAY

MORNING
BIO 1.2 / PCP 1: Process and Co-products of Biofuel and Industrial Production
Chair(s): K. Liu, USDA, ARS, PWA, USA; D. Solaiman, USDA, ARS, ERRC, USA; J. Wanasundara, Agriculture & Agri-Food Canada, Canada; and H. Wang, Iowa State University, USA

Product Opportunities from Algae. Joel Butler, Joanna Money, Solix Biofuels, Ft. Collins, CO, USA

While much of the recent interest in algae has been driven by its value as a potential fuel, algae has the potential to deliver a large number of additional products including nutraceuticals, food supplements, feed for animals and fish and bioplastics. Some of these products are more valuable than biocrude but their extraction from the algae is complex and therefore their viability as a product is still unclear. This presentation will describe these potential products in more detail and discuss some of the challenges facing algae producers in extracting their value.

In situ Transesterification of Algae for the Production of Fatty Acid Methyl Esters for Use as Biodiesel. M.J. Haas, K.M. Scott, Eastern Regional Research Center, USDA, Wyndmoor, PA USA

There has recently been considerable interest in the use of lipids synthesized by algae as feedstocks for the production of renewable fuels. One possibility is the production of biodiesel, which consists of the simple alkyl esters of fatty acids, from algal acylglycerols. This would typically involve the dewatering of the algal biomass, recovery of its lipid by pressing or solvent extraction, and transesterification of the resulting lipid preparation to form biodiesel. An alternative to this approach, termed 'in situ' transesterification, is to conduct the transesterification reaction directly on the dried algal biomass. This eliminates the need for pressing or solvent extraction steps to recover the oil from the algal mass before transesterification, and could therefore simplify and reduce the cost of biodiesel production. We have investigated this approach for the synthesis of biodiesel from algal oil, and will present the results of this investigation.

Glycerine - A Valuable Biodiesel Coproduct for Fermentation Processes. R.D. Ashby, D.K.Y. Solaiman, T.A. Foglia, USDA, ARS, ERRC, Wyndmoor, PA, USA

One of the impediments in the widespread use of biodiesel is the prohibitive cost involved in removing and/or refining the glycerine coproduct. Attempts to impart value to crude glycerine have resulted in the development of a number of technologies designed to utilize crude glycerine as a precursor for value-added product synthesis. The focus of our research group is the bioproduction of green polymers and surfactants with potential as substitutes for petroleum-based materials. We have focused our attention on the utilization of low-value feedstocks (*e.g.* glycerine, soy molasses) to improve process economics of fermentation-based syntheses. In this presentation, we will discuss our continued efforts to improve product yields of polyhydroxyalkanoates (PHA; bacterial polyesters) and sophorolipids (SL; glycolipid surfactants) from both refined and crude glycerine through manipulation of fermentation parameters and the potential for property modification of the bioproducts based on the absolute content of the crude glycerine. Lastly, we have demonstrated the antibacterial properties of SL towards *Propionibacterium acnes* by using SL:Poly-3-hydroxybutyrate $(PHB)_{glycerine}$ composite films and showed, through scanning electron microscopy, the physical effects of SL addition on the surface topography and porosity of PHB polymer films.

Advances in Corn Ethanol Enzyme Technology, Effect on DDGS and Opportunities for Animal Feed Industry. M. Hruby, Danisco Animal Nutrition, Woodbury, MN, USA

Enzymes are crucial in production of ethanol from corn and other starch sources. Enzymes facilitate production of

sugars used by yeast to yield ethanol through a fermentation process. Enzymes can also function as processing aids to improve throughput, reduce slurry viscosity and salt deposits in the processing equipment, water and the use of other compounds such as acids. Ethanol production in United States comes mainly from dry mill grind plants. Besides ethanol and CO_2 , dry mill grind plants also produce distillers dried grains with solubles (DDGS). DDGS are increasingly counted as economically important products of ethanol production process. A deliberate focus on higher nutrient digestibility, low antinutrient levels and high uniformity of DDGS could result in a significant increase of ethanol plant′s revenues. Targeted focus on DDGS quality can be crucial at times when ethanol production gives lower return on investment. Nutritionists have been using greater quantities of DDGS in animal diets. Understanding the effect of various enzymes used in ethanol production process on DDGS quality and uniformity can provide final users, whether domestic or in export markets, with a valuable information to achieve a greatest economic benefit in production of animal protein when offered specific DDGS to include in animal feeds.

Techno-economic Analysis of Brassica Protein and Co-product Extraction Technologies for Food and Biofuel Applications. Edmund K Mupondwa, Janitha Wanasundara, Bioproducts and Bioprocesses, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada

This study presents a techno-economic analysis of an aqueous extraction process to recover proteins of Brassica oilseeds, focusing on napin (2S) and cruciferin (11S) proteins, both of which have distinct biological, physicochemical, and structural properties as well as industrial applications. This is distinct from other processes whose derived protein extracts are mixtures of 11S and 2S proteins. The aqueous extraction process provides various fractions of different physicochemical and functional properties that are expected to significantly enhance high-value application of Brassica proteins, beyond current traditional uses. A mass transfer study of a pilot plant setup was conducted together with an economic analysis of the extraction process. Simulations were conducted for capital equipment sizing and costs to predict various commercial-scale plant capacities. A full economic and financial analysis was completed including capital investment, annual manufacturing costs, primary and co-product revenues, product sales, risk-adjusted return on investment, and payback period, using an appropriate rate of return. The study shows that the economics of the aqueous extraction process are positive notwithstanding the fact that the aqueous extraction operating conditions had not yet been optimized.

Manufacturing New Food Co-Product(s) Using a Novel Ethanol Corn Fractionation System. T. T. Lohrmann, D.J. Hammes, Quality Technology International, Inc, Elgin, IL USA

A majority of the ethanol plants today are designed such that the entire corn kernel is ground prior to fermentation. The remaining non-fermentable fraction after fermentation is commonly dried and sold into the livestock markets as a lower value feed product termed distiller dried grains with solubles. The current focus of our technology is to convert ethanol plants from a traditional dry grind system into a next generation fractionation bio-refinery capable of making bulk food products such as high TDF corn bran and high purity corn germ for further processing to corn oil. Since our wet fractionation systems for ethanol do not utilize sulfur dioxide, production of high purity food products are attainable. Recent work by our group on second generation products has shown that corn germ proteins can be isolated and purified in to novel commercial food products. Other second generation, further-processed food products are in development which will bring even greater consumer benefits and value from this evolving industry.

Identification of Novel Co-product Opportunities From the Low Temperature Fermentation of Grains to Ethanol. D. Bressler, R. Zijlstra, A. Gibreel, University of Alberta, Edmonton, AB, Canada

With the support of the fermentation industry, research focused on determining the biofuel potential of several non-traditional grains including barley, triticale, pulses and other crops with wheat and corn serving as benchmark feedstocks. Research into the fermentation of these non-traditional grains via both traditional jet-cooking and low temperature enzymatic approaches was conducted. An emphasis was placed on freeze-drying the resultant distillers grains and the final product were chemically profiled and assessed in terms of feed value as well as opportunities for the extraction of high value components. Chemical profiling clearly demonstrated that with newer low temperature enzyme approaches, several high-value components survived fermentation and were in fact concentrated three to five fold in the resulting solids fractions. The high-value components identified included tocopherol, tocotrienols,

polyphenols, and phytosterols. Additionally, the fatty acid composition of the grains was preserved through fermentation. The identification of these potential co-products as an extraction opportunity prior to high temperature drying of distillers grains provides an opportunity for diversification of value-addition in the ethanol industry.

The Composition of Crude Corn Oil Recovered after Fermentation via Centrifugation from a Commercial Dry Grind Ethanol Process. R.A. Moreau[1], K.B. Hicks[1], D.B. Johnston[1], N.P. Laun[2], [1]ERRC, USDA, Wyndmoor, PA, USA, [2]Western New York Energy, Medina, NY, USA

A study was conducted to examine the chemical composition of corn oil obtained via centrifugation after fermentation of corn to make fuel ethanol, and compare its composition to that of corn germ oil (commercial corn oil) and experimental corn oils. The levels of free fatty acids in the post fermentation corn oil were high (13-16%), as previously reported. The levels of free phytosterols and hydroxycinnamate steryl esters (similar to oryzanol in rice bran oil) were higher than those of corn germ oil and were comparable to those of ethanol extracted corn kernel oil. The levels of tocopherols and tocotrienols were lower in post-fermentation oil than in either corn germ oil or ethanol extracted corn kernel oil. The levels of lutein and zeaxanthin in post-fermentation were much higher than those in corn germ oil and were comparable to those in ethanol extracted corn kernel oil. Overall, exposure to all upstream processes of a fuel ethanol plant, including high-temperature liquefaction, saccharification and fermentation appeared to have the greatest effect on tocopherols and tocotrienols, but it had little effect on the levels of free phytosterols, hydroxycinnamate steryl esters, lutein and zeaxanthin.

Changes in the Oil Concentration, Fatty Acid Composition, and Functional Lipid Profiles During Dry Grind Ethanol Production from Corn. Keshun Liu[1], Robert Moreau[2], Jill Moser[3], [1]USDA, ARS, Aberdeen, ID, USA, [2]USDA, ARS, ERRC, Wyndmoor, PA, USA, [3]USDA, ARS, NCAUR, Peoria, IL, USA

Demand for alternatives to fossil fuels has resulted in a dramatic increase in ethanol production from corn. The dry grind method has been the major process, resulting in a large volume of dried distiller grains with solubles (DDGS) as a co-product. This presentation reports our study to monitor concentration changes of various types of lipids during the entire dry grind process of corn. Samples of ground corn, intermediate products and DDGS were provided by three commercial plants in Iowa. After lyophilization, the moisture and crude oil levels as well as the fatty acid, phytosterol, tocopherol, and tocotrienol composition were measured. Results from Plant 1 samples show that the three steps that caused significant increases in oil content as compared with ground corn were slurrying, fermentation, and centrifugation (only into thin stillage). However, for all other lipids measured, including fatty acid composition (relative to total fatty acids), and composition of phytosterols, tocopherols and tocotrienols (relative to total lipid mass), the process did not cause significant changes, even at the three critical steps that caused significant increases in oil content. The conclusion is that the dry grind process caused a significant change in lipid quantities, but not lipid quality. Data from Plant 2 and 3 confirmed those found with Plant 1 samples.

Changes in Physical Properties During Dry Grind Processing of Corn. K.A. Rosentrater[1], K. Liu[2], [1]USDA, ARS, Brookings, SD, USA, [2]USDA, ARS, Aberdeen, ID, USA

Corn-based ethanol has dramatically increased in the U.S. in recent years. So too has the quantity of coproducts. These are composed of nonfermentable components (i.e., protein, lipid, fiber, and ash) from the corn kernel. These materials are separated from the ethanol and then subjected to various separations and drying processes. The most common coproduct is distillers dried grains with solubles (DDGS). DDGS has become widely used as a livestock feed ingredient. One of the key issues associated with DDGS is poor flowability. The objective of this study was to identify where in the manufacturing process stickiness is imparted to the coproduct material. Eight samples were collected from two corn-based fuel ethanol plants, and included raw corn, cooked slurry, liquefied mash, whole stillage, thin stillage, wet cake, distillers dried grains (i.e., no added solubles), and DDGS. Each of these samples was dried at 50°C for 24 h, then milled to 0.5 mm. Properties tested included Carr Testing (angle of repose, aerated bulk density, packed bulk density, compressibility, angle of spatula, total flowability index, angle of fall, angle of difference, dispersibility, floodability index), thermal conductivity, thermal diffusivity, and color (Hunter L, a, b). Results of these tests will be presented and compared, and implications for the industry will be discussed.

Integrated Production of Ethanol and Succinic Acid in a Biorefinery. Nhuan P. Nghiem, Kevin Hicks, David Johnston, Eastern Regional Research Center USDA ARS, Wyndmoor, PA, USA

Production of succinic acid from glucose by Escherichia coli strain AFP184 was studied in a batch fermentor. The bases used for pH control included NaOH, KOH, NH4OH, and Na2CO3. The yield of succinic acid without and with carbon dioxide supplied by an adjacent ethanol fermentor using either corn or barley as feedstock was examined. The carbon dioxide gas from the ethanol fermentor was sparged directly into the liquid media in the succinic acid fermentor without any pre-treatment. Without the CO2 supplement, the highest succinic acid yield was observed with Na2CO3, followed by NH4OH, and lastly by the other two bases. When the CO2 produced in the ethanol fermentation was sparged into the media in the succinic acid fermentor, no improvement of succinic acid yield was observed with Na2CO3. However, several-fold increases in succinic acid yield were observed with the other bases, with NH4OH giving the highest yield increase. The yield of succinic acid with CO2 supplement from the ethanol fermentor when NH4OH was used for pH control was equal to that obtained when Na2CO3 was used, with or without CO2 supplementation. The benefit of sparging CO2 from ethanol fermentation on the yield of succinic acid demonstrated the feasibility of integration of succinic acid fermentation with ethanol fermentation in a biorefinery.

Assessment of a High Purity Zein Product from Commercial Zein. David J. Sessa, Kristen Kruger Woods, Plant Polymer Research, NCAUR, USDA, ARS, Peoria, IL, USA

Successful utilization of commercial zein products for food, pharmaceutical, cosmetic and medical applications requires a decolorized/deodorized zein that is substantially undenatured protein. A zein protein with those qualifications has already been developed by a patent pending process. The objective of this presentation is to devise methodologies to assess the required attributes. Zein purity was assessed by FTIR and circular dichroism (CD) of commercial zein before and after further purification. Spectral differences were observed in the amide I (1650 cm^1^) peak, amide II region (1530 and 1515 cm^1^) and the amide III peak at 1240 cm^1^, where ratios of dominant peaks were strongly dependent on purity of sample. CD analyses validated FTIR results by showing increased α-helical content for purified zein. We defined off-odor removal by UV spectroscopic ratio of 280nm:325nm of $\geq$8, where diferoylputrescine is the major contributor. Color removal, attributed to xanthophylls in zein was followed by a visible spectroscopic assay. These results are a major contribution for achieving a consistent zein product.

AFTERNOON

BIO 1.2 / PCP 1: Process and Co-products of Biofuel and Industrial Production

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

Proteins as Renewable Flocculants. G. Piazza, R. Garcia, ERRC, ARS, USDA, Wyndmoor, PA, USA

Nine billion chickens are slaughtered in the U.S. per year, but the protein rich chicken blood (CKB) is mainly treated as waste. We have investigated the use of CKB fractions as replacements for a widely used nonrenewable, polymeric flocculant, anionic polyacrylamide (PAM), which requires the addition of calcium chloride for activity. PAM is needed for several municipal and industrial operations. The CKB frozen fractions studied were (A) blood, freed of coagulated blood; (B) blood plasma (blood centrifuged to remove blood cells); and (C) heated blood, centrifuged to remove solids. These CKB fractions were dehydrated two ways: freeze drying and spray drying at elevated temperature. Flocculation activity was quantified by determining the concentration of flocculant needed to settle a constant amount of suspended clay particles in 1 h, 5 h and 24 h. Flocculation activity by the CKB fractions required the addition of buffer, citric acid, phosphoric acid or sulfuric acid to reduce the pH to 5.5, but does not require the addition of calcium chloride. The flocculation activity of frozen and freeze dried CKB fractions was superior or equivalent to that of anionic PAM. The flocculation activity of spray dried fractions A and B was nearly as good as that of anionic PAM. The results indicate that CKB fractions may be viable renewable replacements for PAM.

PCP 2: Ground Corn Meal in Ethanol Industry - Food Grade Applications

Chair(s): T. Yunusov, NFI Iowa, USA; and R. Aluko, University of Manitoba, Canada

Corn Ethanol - Backend Fractionation The Solution is in Solution. David Winsness, GreenShift Corporation, Alpharetta, GA, USA

GreenShift Corporation develops and commerciallizes clean technologies designed to integrate into and leverage established production infrastructure to address the financial and environmental needs of its clients.This presentation targets corn ethanol producers and GreenShift's portfolio of backend fractionation technologies. GreenShift has pioneered the backend fractionation process and has recieved patents for some of its corn oil extraction methods. Deploying GreenShift's corn oil extraction technologies into a dry mill ethanol facility is widely considered to be the quickest path to margin improvement for first generation corn ethanol producers today.The corn oil extraction technologies increase biofuel yields per bushel of corn by 7% while reducing energy and greenhouse gas (GHG) intensity of corn ethanol produciton by more than 21% and 29%, respectively. These benefits correspond to increased ethanol producer incomes by about $.12 per gallon of ethanol produced at current market prices, and can be realized for less than 10% of the capital cost of the host facility.Corn oil oil extraction is only the beginning of a series of technologies within GreenShift's Backend Fractionation portfolio and these technologies are designed to increase sustainability and global competitiveness of the corn ethanol industry.

New Co-product Production from Fuel Ethanol Processing Streams. D.B. Johnston, A.J. McAloon, United States Department of Agriculture, ARS, ERRC, Wyndmoor, PA, USA

Distillers dried grains with solubles (DDGS) and carbon dioxide are currently the only coproducts produced from the corn dry grind ethanol process. The DDGS are sold for use in ruminant animal diets and to a much lesser extent for use in other animals. The development of alternative processes for new coproducts and improved energy utilization have been long term research goals in our laboratory. Over the last few years we have been researching the feasibility of co-production of value added products from ethanol processing streams prior to the DDGS being produced. Several strategies were investigated and will be discussed. Utilizing the thin stillage processing stream, we have been able to grow Phaffia rhodozyma, an aerobic yeast, which can produce the high value carotenoid astaxanthin. Astaxanthin is used in aquaculture and is the natural pigment responsible for the flesh pigmentation of salmon. We have produced an integrated process and cost model for the production of astaxanthin containing feed ingredient from the thin stillage, utilizing data generated from laboratory fermentations. Utilizing a similar strategy, we propose several other coproducts from corn to ethanol processing streams.

New Corn Degerming Processes and the Germ Quality. Hui Wang[1], Tong Wang[2], Lawrence Johnson[1,2], [1]Center for Crops Utilization Research, Iowa State University, Ames, IA, USA, [2]Department and Human Nutrition, 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.

The Processing of Corn for Food, Feed, and Energy. G. Haider, C. Teeter, Crown Iron Works Company, Roseville, MN, USA

Value added processing streams in the Ethanol Industry. Focusing on de-germination of the whole corn kernel prior to fermentation, followed by up-front oil extraction and post oil extraction to produce feed and additional fuel source material. Covering processes that allow the ethanol plant to maintain ethanol production, while also producing by products for the food and feed industry. Offering an argument to the statement food for fuel.

Corn Processing to New Food Ingredients - Corn Oil Powder. R. Barton, NFI Iowa LLC, Osage, IA, USA

Dry ground corn flour is a raw material for approximately 80% of the ethanol plants in the USA. The main idea developed in the presentation is a two-step process to get food grade corn products and modified starch for existing

ethanol technologies. The food grade products include practically all corn constituents except the starch. The stream-modified starch is partly hydrolyzed corn starch which can be used directly for the fermentation process during ethanol production. Some commercial aspects of the approach will be discussed.

TUESDAY

AFTERNOON

PCP 3: Proteins for Healing: From Peptides to Macromolecules

Chair(s): H. Ibrahim, Kagoshima University, Japan; and H. Kumagai, Nihon University, Japan

Lunasin Reduces Colon Cancer in vitro by Modifying the Expression of Clusterin Isoforms to Promote Cell Death. V. Dia, E. Gonzalez de Mejia, University of Illinois, Illinois, USA

Lunasin is a naturally occurring chemopreventive peptide with unique amino acid sequences responsible for its anti-cancer properties. Clusterin is an apolipoprotein implicated in several diverse physiological processes and its expression is associated with contradictory functions either tumor progression, resistance to treatment in vivo, cell survival or apoptosis associated to its different isoforms. The objective of this study was to assess the potential of lunasin to reduce resistance of human colon cancer cells to cell death by modifying the expressions of different clusterin isoforms. Our data showed that the expression of the pro-apoptotic nuclear clusterin (nCLU) in HT-29 human colon cancer cells increased from 8% to 44% when treated with 10 μM lunasin. This increase in the expression of nCLU was accompanied by increase in the expression of p21, reduction in the number of viable HT-29 cells and increase in the number of HT-29 cells undergoing apoptosis. Moreover, HT-29 cells were transfected with the anti-apoptotic form of clusterin known as secretory clusterin (sCLU), associated to development of resistance to cell death, and the effect of lunasin on the expression of sCLU was measured. In summary, lunasin promoted cell death in colon cancer cells by modifying the expression of different clusterin isoforms.

Bowman-Birk Inhibitor: Bioactivities of a Small, Soluble Soybean Protein. C. Schasteen, Solae LLC, Saint Louis, MO, USA

Soy has been consumed safely for many hundreds if not thousands of years. Soybeans in many forms, whole, or processed into milk, or as isolated protein fractions has also been consumed safely for generations by millions of humans. Several protease inhibitors, Bowman-Birk and Kunitz, most commonly referred to as trypsin inhibitors BBI and KTI, respectively, are the best known and the most studied biologically active proteins in soybean. KTI is the acknowledged antinutrient and is inactivated using heat. BBI is not an antinutritive, is heat stable and has been reported in the literature to possess diverse bioactivities including anticancer, anti-inflammatory, positive effects in neuromuscular disease, ulcerative colitis, radiation-induced transformation *In vitro* and a decrease in the photoaging of skin. The soybean BBI molecular weight calculated from the amino acid sequence of 71 residues is 7975 Da, however, multiple isoforms are known to exist. All BBI's have 2 different exposed peptide loops which interact with trypsin and chymotrypsin and are known to self-associate into dimers and higher multimers. The molecular targets for the variety of bioactivities noted above are not completely understood. Examples of the different bioactivities of BBI will be presented.

Novel Protein-based Drug-targeting Strategy from Beneath the Shell of Egg. Hisham Ibrahim, Kagoshima University, Faculty of Agriculture, Kagoshima, Japan

Antibiotic resistance is a problem that continues to challenge the healthcare sector and is foremost stressing in the management of infectious diseases. New therapeutic strategies are needed to address this challenge. Although the therapeutic efficacy of new drugs has been established, inefficient delivery to the target cell can result in inadequate therapeutic index. Especially, many intracellular infections are difficult to treat because of the poor solubility of the antimicrobial drugs and their poor cellular transport leads to concentrations inside the cells below the minimum inhibitory dose. One strategy to overcome this problem is to load efficient drugs into a safe protein carrier that can specifically recognize and then deliver the drug into the target microbial cells. Our structural analysis of egg proteins

show great promise for engineering a range of new GRAS (Generally Recognized As Safe) carriers with the potential to incorporate pharmaceutical compounds and provide a novel food-protein-based drug targeting systems. In this study, I will introduce our novel strategy of egg protein-based antimicrobial drug delivery system that can specifically deliver hydrophobic drugs with poor solubility to the pathogenic bacterial cells in a targeted manner, which offer tremendous opportunities for the treatment of infectious diseases.

Preparation of Fermented Egg Sauce by Use of Delipidated Egg Yolk. Hajime Hatta[1], Sakiko Shou[2,1], Yoshie Ueno[3], [1]Kyoto Women's University, Kyoto, Japan, [2]Hishiroku Co., Ltd., Kyoto, Japan, [3]Kyoto Prefectural Technology Center for Small and Medium Enterprises, Kyoto, Japan

Soy sauce is made from koji with salted water during fermentation for about 6 month. The soy koji is an indispensable material in which Aspergillus oryzae was cultured on mixture of defatted soybeans with roasted wheat grains. In recent years, yolk lipid has been used as a source of bioactive fatty acids (AA and DHA) for an infant formula. An increasing demand of using yolk lipids has resulted in remaining surplus delipidated yolk.In this report, we cultured egg koji by using delipidated yolk instead of using defatted soybeans. The yolk koji obtained showed about 2-3 times higher protease activities than that of the soy koji. Then, each soy or egg koji was fermented with salted water to make soy sauce or egg sauce, respectively. The umami taste and flavor of the egg sauce were superior to that of the soy sauce after 4 months fermentation. HPLC profile indicated that only amino acids and oligopeptides were detected in egg sauce, whereas considerable amount of proteins were remained in soy sauce. Moreover, color of the egg sauce was much lighter than that of the soy sauce. It was revealed first time that delipidated egg yolk is a suitable material for growth of Aspergillus oryzae to produce a novel characteristic seasoning, egg sauce, as well as novel peptides originated from egg yolk proteins.

Cedar Pollen Cry j 1-galactomannan Conjugate Can Be Used as an Immunotherapy for Pollinosis Pacients. Akio Kato[1], Rieko Aoki[1], Akira Saito[2], Hiroyuki Azakami[1], [1]Yamaguchi University, Yamaguchi, Japan, [2]Wako Filter Technology, Tokyo, Japan, [3]Department of Biological Chemistry

We have reported that the oral administration of major cedar pollen allergen Cry j 1-galactomannan conjugate causes the remarkable suppression of cedar pollinosis. In order to evaluate the effect of various attached saccharides on the masking of allergenic epitopes and the transport into gut lumen to phagocytose in immune cells, various saccharide-allergen Cry j 1 conjugates were prepared. Major allergen of Japanese cedar, Cry j 1 was conjugated with galactomannan , dextran, xyloglucan, and various mono-saccharides through Maillard reaction by dry-heating. The Cry j 1-galactomannan conjugate completely masked the epitopes of allergen in Cry j 1. The small size of oligo-saccharide and various momosaccharides cannot mask the epitope of allergen Cry j 1. This suggests that the high molecular size of attached saccharides is important to mask sterically the epitope sites. Cry j 1-galactomannan conjugate and Cryj 1-mannose conjugate were effectively trafficked into gut lumen and phagocytosed by the immune cell such as dendritic cell in gut. This result suggests that Cry j 1-saccharide conjugates are phagocytosed via mannose receptor in the immune cell. These results indicate that the most suitable saccharide to conjugate with allergen Cry j 1 is galactomannan to completely mask the epitope site and to phagocytose in gut.

Development of the Molecules for Antigen-specific Immune Tolerance against Cedar Pollinosis. A. Saito[1,2], H. Kageshima[1], S. Hirano[1], R. Aoki[2], A. Kato[2], [1]Biobusiness Propulsion Division, WAKO FILTER TECHNOLOGY Co.,Ltd., bando-shi, Ibaraki, Japan, [2]Protec Co.,Ltd., Yamaguchi University, Yamaguchi-shi, Yamaguchi, Japan

About 30% of Japanese are suffering from the pollinosis of cedar pollinosis in early spring. However, no effective therapeutic drugs to reduce or cure fundamentally the symptom have not been developed. We found that desensitization therapies using major pollen allergen (Cry j 1)-galactomannan (GM) conjugate are effective to reduce the allergenic symptom. When Cry j 1-GM conjugate was orally administrated for one month before flying of cedar pollen, most patients (70-80%) are significantly recovered from pollinosis without any anaphylaxis. To elucidate the molecular mechanism of the effective oral immune tolerance, the activation of gut immune cells, CD11c+dendrite cell (DC) and CD4+ CD25+ FoxP3+ T cell (Treg) were investigated by histological and flow cytology studies using allergen-sensitized mice. Histological study shows that the absorption into gut was exclusively observed in the Cry j 1-

GM conjugates, suggesting that the allergen-polysaccharide conjugate was effectively uptaken into the gut and was phagocytosed by DC. On the other hand, the flow-cytology analysis of immune cells showed remarkable increases in the population of regulatory T cell (Treg), suggesting remarkable decreases in IgE production. These data suggests that immune-tolerance using allergen Cry j 1-GM conjugate can be used as a therapy of pollinosis.

Is Wheat-dependent Exercise-induced Anaphylaxis Induced by Gli-B1?. T. Nagano[1], M. Tanaka[1], Y. Nakayama[1], T. Nezu[1], H. Yano[1], Y. Kato[1], T. Matsuda[2], T. Ikeda[3], K. Haruma[4], [1]Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan, [2]Nagoya University, Nagoya, Aichi, Japan, [3]Agricultural Research Center for Western Region, Fukuyama, Hiroshima, Japan, [4]Kawasaki Medical School, Kurashiki, Okayama, Japan

Food-dependent exercise-induced anaphylaxis is a distinct form of food allergy induced by physical exercise. Wheat is reported to be the most frequent allergenic food in Japan, and its symptom is called wheat-dependent exercise-induced anaphylaxis (WDEIA). Although Gli-B1 was identified as a cause of WDEIA, the mechanism by which Gli-B1 induces WDEIA remains unclear. In this study, we purified Gli-B1 and investigated the effect of Gli-B1 on WDEIA in a mouse model. Gli-B1 was purified from soft wheat flour. The purified Gli-B1 were identified as three bands around 60 kDa in SDS-PAGE and the N-terminal amino acid sequences of these three bands all were â??SRMLSPRGKE' that was identical to Gli-B1. B10.A strain mice were sensitized by interperitoneal injection with gliadin/alum. The serum of the sensitized mice was analyzed for specific IgE antibody by enzyme-linked immunosorbant assay. The Gli-B1-specific IgE was detected highly in the sensitized mice serum. To assess anaphylactic reaction in the sensitized mice, body temperature and voluntary physical activity were determined. The decreased in the body temperature and the voluntary physical activity of gliadin-sensitized mice were observed after Gli-B1 challenging. It is noteworthy that Gli-B1 induces allergenic symptom without exercise under the condition that gliadin does not.

The Anti-fatigue Effects of Salmon Muscle Extract Containing Anserine. Hiroaki Honda, Yoshinori Takahashi, Masataka Kawarasaki, Hirokazu Muneda, Masahiro Sugimoto, Hiroyuki Enari, Central Research Institute, Maruha Nichiro Holdings,Inc., 16-2,Wadai,Tsukuba-City,Ibaraki,300-4295 Japan

Migratory fish and birds have rich anserine in their muscle to maintain stamina and instantaneous power. We evaluated the anti-fatigue effects of the salmon muscle extract containing anserine (SEAns) in healthy male subjects. The SEAns inhibited the creatine phosphokinase activity and suppressed the elevation of the cortisol level in blood. In addition, it improved endurance exercise. From these results, we showed that the SEAns had the reductive effects on physical fatigue and mental stress. On the other hand, it is said that physical fatigue and mental stress are closely related to asthenopia. So, we evaluated the effects of SEAns on asthenopia in visual display terminal (VDT) workers. Forty VDT workers, who complained of asthenopia, were divided into three groups. They took SEAns (0, 0.4, 1.3 g/day, respectively) everyday for eight weeks. The subjects who took 0.4 g and 1.3 g of SEAns felt remarkable effects on asthenopia from the 4th week. Moreover, the near point of accommodation of the SEAns group was improved significantly compared to the placebo group. In addition, we evaluated the effects of SEAns on presbyopia recently. SEAns improved significantly the near point of accommodation in presbyopia. These results indicated that the SEAns might have the effects on asthenopia and presbyopia.

Influence of Rat Strain and Diet Composition on Dietary β-Conglycinin-Dependent Reduction of Food Consumption and Modulation of Lipid Metabolism. K. Koba[1], D. Oikawa[1], S. Tamaru[1], K. Tanaka[1], M. Sugano[2], [1]University of Nagasaki, Siebold, Nagayo, Nagasaki Japan, [2]Professor Emeritus, Kyushu University, Fukuoka, Japan

We previously observed that feeding of β-conglycinin (CON) as compared with casein (CAS) and soy protein (SOY) decreased the body weight gain in rats, due to a significant decrease of food consumption. Since the CON-dependent decrease of food consumption was not always found in published data, we examined whether the CON-dependent effect was influenced by diet composition (AIN-93G or AIN-76) and/or rat strain (Sprague Dawley (SD) or Wistar). Male SD or Wistar rats were fed the diets prepared according to AIN-93G or AIN-76 formulas, containing 20% protein; CAS, SOY, CON, or 1:1 mixture of CAS and CON (CAS+CON). After 4 weeks feeding period, dietary CON as compared with CAS and SOY, decreased food consumption and hence, body weight gain, irrespective of the diet formula and the strain of rats. The findings suggested that decreased food consumption by feeding of the CON diet

could be independent on the diet formula and rat strain but dependent on the protein per se. The CON-dependent decrease of food consumption was not observed in rats fed the CAS+CON diet at all. However, in rats fed this combined diet, CON-dependent physiological effects were still kept at the significant level such as decreases of visceral adipose tissue weight and serum triglyceride concentration.

WEDNESDAY

MORNING

PCP 4: Novel Technologies for Isolation and Extraction of Proteins and Co-Products

Chair(s): P. Kerr, Solae Co., USA; and S. Jung, Iowa State University, USA

Aqueous Extraction of Oil and Protein from Soybeans by Subcritical Water Treatment. Sipho C Ndlela[1], Juliana Maria Leite Nobrega de Moura[2], Lawrence A. Johnson[2], Norman K. Olson[1], [1]Iowa State University, Iowa Energy Center, BECON, Nevada, IA, USA, [2]Iowa State University, Center for Crops Utilization Reasearch, Food Science Department, Ames, IA, USA

The effects of solids-to-liquid ratio (1:3.3 - 1:11.7), temperature (66 - 234°C), and extraction time (13.2 - 46.8 min) were evaluated on the extraction of oil and protein from soybean flakes and extruded soybeans flakes by subcritical water treatment. Central composite design (23), with three center points and six axial points, was used. Subcritical water extractions of oil and protein were carried out in a 1L high pressure stainless-steel batch reactor with constant stirring (300 rpm) at 5-560 psi. In general, higher oil extraction was obtained using extruded soybean flakes compared with soybean flakes. Oil extraction from extruded flakes improved when using temperature around the central point (150°C) while oil extraction from flakes was not significantly affected by the variables in the evaluated range. Protein extraction from extruded flakes improved when increasing SLR and temperature, with higher extraction yields at temperatures above 150°C. Improved protein extraction from flakes was observed when temperature moved away from the central point (150°C) and SLR increased. Protein extraction yields from flakes were generally higher than obtained from extruded flakes, especially when using low temperatures (66-100°C) and SLR ranging from 1:7.5 to 1:10.

The Twin-screw Extrusion Technology: An Original Solution for the Extraction of Proteins from Sunflower and Alfalfa. Philippe Evon[1,2], Dorothée Colas[1,2], Pierre-Yves Pontalier[1,2], Luc Rigal[1,2], [1]Université de Toulouse, INP, LCA, ENSIACET, Toulouse, France, [2]INRA, LCA, Toulouse, France

Twin-screw extrusion has been used for the protein extraction from sunflower and alfalfa. Thermo-mechanical fractionation and aqueous extraction are conducted simultaneously to collect separately a liquid extract and a solid raffinate.From sunflower whole plant, squeezing in the reversed screws is favored by the fibers abundance in the stalk, and it enables L/S separation. Protein yield is 44%, in the best conditions, and lipids are partly co-extracted. Water-soluble proteins are in an aqueous extract and in two O/W emulsions due to their surface-active properties. Hence, the oil is co-extracted in the form of emulsions stabilized by proteins at interface.Proteins can be collected in the aqueous extract by isoelectric precipitation. Emulsions are usable for oil production. Their demulsification with ethanol produces a precipitate rich in proteins with low denaturation level.From alfalfa whole plant, the highest protein yield in the aqueous extract (called green juice) is 25% when water is added in the extruder. Water-soluble and water-insoluble green juice proteins can be fractionated thanks to L/L extraction in sunflower oil and ammonium sulfate precipitation.Some other molecules are co-extracted (polysaccharides, phenolic compounds). They can be purified with ultrafiltration or chromatography.

A New Modular Concept to Produce Proteins. F. Pudel[1], R.-P. Tressel[1], G. Börner[2], [1]Pilot Pflanzenöltechnologie Magdeburg, Magdeburg, Germany, [2]ÖHMI Engineering GmbH, Magdeburg, Germany

There is a worldwide growing market for plant proteins. Animal proteins will be partially or completely replaced by proteins of vegetable origin in certain applications in the coming few years. Considering the raw material shortages and demands in functionality new plant protein sources are needed with a high reliability in both supply and quality.

Rapeseed meal is such a specific source for highly-functional proteins. Besides the cruciferin (globulin) fraction, the ordinary storage protein of all oilseeds, only rapeseed contains a high amount of napin (albumin) - a low molecular protein with unique properties. An adapted and flexible processing is needed to extract rapeseed proteins with variable qualities for different market segments.A modular concept is presented which offers the possibility to produce rapeseed protein concentrates and isolates. The process is based on a new quality of the rapeseed meal realized by a fluidized bed desolventizer system. After an aqueous separation of the various meal fractions (fibre concentrate, protein-rich cellular material, proteins in solution) the user can adjust the kind of end product by a flexible after treatment of the fractions.First results on protein quality and functionality are discussed as well.

Fractionation of Storage Proteins of *Brassicaceae* Oilseeds. J. Wanasundara, T. McIntosh, Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada

Oil bearing seeds of *Brassicaceae* family play a major role in Canadian edible oil and biofuel oil industries. Removal of oil leaves behind seed storage protein rich oilseed meal. Seed storage proteins of *Brassicaceae* oilseeds are mainly cruciferins (11S globulin) and napins (2S albumin) that are different in molecular size, structural organization, physico-chemical properties and biological activities. Therefore obtaining these proteins separately will enable to maximize the uses based on Brassicaceae oilseed meal proteins. Detail studies on solubility characteristics of napin and cruciferin revealed unique differences of these proteins. A simple process was developed to obtain these two proteins based on solubility characteristics. This process uses simple unit operations, minimum chemical inputs and is easily scalable. All products obtained from this process can be used in either food or non-food applications.

Plant Proteins - A Clear Solution for Beverages. Martin Schweizer, Kevin Segall, Sarah Medina, Brent Green, Burcon NutraScience (MB) Corp., Winnipeg, Manitoba, Canada

Plant proteins present an excellent alternative to animal proteins for use in various processed foods and beverages. The use of plant proteins in acidic applications, such as ready to drink or powdered acidic beverages, has traditionally been hindered by the limited solubility of the protein ingredients. Burcon NutraScience has developed and patented processes for the production of plant protein isolates that are completely soluble in an acid environment. The low pH solutions of these isolates are both transparent and heat stable. Burcon has developed plant protein isolates from both rapeseed/canola (Supertein™ and Puratein®) and soy (Clarisoy™). ClarisoyTM is 100% soluble and completely transparent in acidic solutions, enabling its use in acidic beverages with pH values ranging down to 2.5 and lower. Such low pH solutions of Clarisoy™ are heat stable, permitting thermal processing, such as hot fill. Clarisoy™ also lacks the traditional ?beany? taste of soy giving beverages a cleaner flavor. The solubility, transparency and clean flavor of Clarisoy™ allow for its use in a wide variety of beverages where traditional soy protein isolates are not appropriate. In addition, Clarisoy™ shows great promise for use in a variety of other food applications. Supertein™ is a canola protein isolate with functional properties including excellent solubility, the ability to form transparent solutions and foaming. Supertein™ solutions retain their clarity when heated at low pH. Applications for Supertein™ include beverages, confectionery, aerated desserts, and protein bars, among many others. Nutritionally, Supertein™ has an excellent amino acid profile, being uniquely rich in sulfur amino acids, particularly cysteine. The typical cysteine content of Supertein™ is nearly double that of whey protein, which is recognized for its high cysteine content. Recent research has suggested that a high content of dietary cysteine may play a major role in the prevention of heart disease and diabetes. As such, Supertein™ has excellent potential as a functional food ingredient. Puratein® is a canola protein isolate with functional properties that include emulsification, gel formation, thickening, formation of heat-stable foams, and water- and ingredient binding. Applications for Puratein® include dressings, meat substitutes, baked goods and protein bars, among many others.

Development and Implementation of a Novel Integrated Hexane-free Process for the Isolation of Oil and Soluble Protein from Canola-grade *Brassica Juncea*. J. Doucet[1], G. Beye[2], N. Tang[3], L. Rozenszain[4], [1]Kengtek Engineering, 685 Bl. Ste Foy, Longueuil, QC, J4J 1Z1, Canada, [2]Bioexx Proteins of Saskatoon Inc., 33 Peters Ave., North Corman Ind. Park, site 404, Saskatoon, SK, S7K 3J7, Canada, [3]POS Pilot Plant Corp., 118 Veterinary Road, Saskatoon, SK, S7N 2R4, Canada, [4]BioExx Specialty Proteins Ltd., 219 Dufferin Street Suite 100B, Toronto, ON M6K 3J1, Canada

Purification of protein hydrolysates and isolates from canola first requires efficient removal of the oil through pressing and solvent extraction of the residual oil. In order to retain maximum functionality in the isolates, thermal damage should be limited as much as possible throughout the oil recovery processes. An alternative solvent extraction and recovery process is here presented that in combination with cold pressing recovers the oil from the seed at much lower temperatures than traditional hexane extraction. Press cake containing 18%-24%(w/w) residual oil is suspended in a novel low-boiling point solvent system allowing temperatures to be kept below 60 C throughout the process. Extraction of the protein and development of isolates is then initiated without the traditional desolventization stages resulting in protein isolates of exceptional solubility and functionality. Integrating recovery of insoluble proteins as higher value products allows maximization of protein value of the seed.As a starting point for the design, we will present experimental results showing the oil solubility isotherms as a function of the solvent composition. From there, experimental results in lab and pilot scale extractors will be presented that will outline the specific mass transfer limitations between the solid and liquid phase. From this work, a reactor model will be presented and compared to experimental data obtained in intermediate scale plant. Finally, the model will be used for scale-up purposes and compared to actual full size plant results.The impact of the solvent system on the protein extraction and the functionality of the resulting proteins will be also discussed.

Phosvitin Extraction from Leftover Egg Yolk. J. Ren, J. Wu, University of Alberta, Edmonton, AB, Canada

Egg yolk phosvitin is one of the most highly phosphorylated proteins in nature. About half of its amino acids are serine, and 90% of these serine are phosphorylated. The unique structure enables phosvitin with unique biological properties, such as metal-chelating, bactericidal and antioxidant activity. Various methods reported involving organic solvents for lipid removal followed by salt extraction and precipitation, or DEAE cellulose chromatography; but most of them are laborious and time consuming, and involve organic solvents or other non food-grade chemicals. The objective of this project was to develop a method of phosvitin extraction without using organic solvent from leftover egg yolk after antibody extraction. Leftover egg yolk is the residue egg yolk in antibody preparation. Egg yolk was diluted by 10 times of water and leftover egg yolk was obtained as pellet after removal water-soluble proteins, which contain antibody. The leftover egg yolk pellet was suspended in aqueous solution to remove lipids; phosvitin was then extracted and precipitated in aqueous salt solution, and further purified by ion exchange chromatography. An environmentally-friendly method of phosvitin extraction was developed which is highly expected in developing valuable functional food ingredients.

Inverse Engineering: Reconstitution of Rapeseed Oleosomes with Native Tensioactives. J.F. Fabre[1], G. Vaca-Medina[1], M. Deleu[2], R. Valentin[1], Z. Mouloungui[1], [1]Université de Toulouse - UMR1010 Chimie Agro-industrielle, ENSIACET, INPT, INRA, Toulouse, France, [2]Unité de Chimie biologique industrielle, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium

Vegetable oleosomes are self-organized oil-bodies that can form micrometric emulsions when dispersed in water. These solutions can then be used in several applications: lubricants, cosmetics?The stability of these emulsions is primordial to assure their industrial potential. Inverse engineering, consisting in the reconstitution of the emulsion with its main components, allowed us to study the role of the membranous molecules (oleosins and phospholipids) on the emulsion stability. We showed that native ratio phospholipids/proteins induce a fragile equilibrium between repulsive (electrostatic, steric) forces and attractive ones (hydrophobic, Van der Waals?).Zeta potential, surface/interfacial tension, size measurements but also freeze/thaw destabilisation tests allowed us to understand how the control of environmental factors, such as pH and the presence of contaminant proteins was essential to create a positive synergetic effect between oleosins and phospholipids so that both flocculation and coalescence rate be minimized.

Synthesis and Characterization of Methoxy Derivatives of Gossypol. M. Dowd[1], C. Zelaya[2], E. Stevens[2], S. Pelitire[1], J. Mellon[1], [1]Southern Regional Research Center, New Orleans, LA, USA, [2]Dept. of Chemistry, University of New Orleans, New Orleans, LA, USA

A series of new 6-methoxy and 6,6′-dimethoxy gossypol derivatives have been synthesized and characterized. A mixture of gossypol, 6-methoxygossypol, and 6,6′-dimethoxygossypol was obtained by acetone extraction of cotton

roots, and a series of known gossypol reactions were carried out on the mixture to form the corresponding methoxy derivatives. Although modification of reported procedures was required, methylated gossypolone, apogossypol, and apogossypolone derivatives were successfully produced. The mixed products were separated by preparative reverse-phase HPLC and isolated by extraction into ether, water washing to strip residual solvent and buffer, and precipitation either from ether or single-phase solutions of ether, water, and acetic acid. Products were characterized for carbon-hydrogen content and melting point, and the UV-vis, 1H-NMR, and mass spectra were determined. Preliminary testing of the isolated starting compounds (i.e., racemic and chiral forms of gossypol, 6-methoxy-gossypol, and 6,6′-dimethoxy-gossypol) and the methoxy-gossypolone forms indicates that gossypolone has the strongest anti-fungal effects.

AFTERNOON

PCP 5: General Protein and Co-Products

Chair(s): N. Deak, Solae Co., USA; and P. Qi, USDA, ARS, ERRC, USA

High Fischer Ratio Multifunctional Peptides from Flaxseed Protein. C.C. Udenigwe[1], R.E. Aluko[1,2], [1]Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada, [2]The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada

Enzymatic protein hydrolysates have exhibited several physiological activities relevant to human health sustenance. In this study, hydrolysis of flaxseed proteins using thermolysin followed by pronase, and mixing with activated carbon yielded a peptide mixture with a Fischer ratio (branched-chain amino acids/aromatic amino acids) of 23.65 and a Phe/Tyr content of 1.11%. Gel permeation chromatography showed that the mixture contained mostly low molecular weight peptides (<4 kDa). The high Fischer ratio peptide sample exhibited antioxidant property by scavenging 2,2-diphenyl-1-picrylhydrazyl radical, superoxide radical and hydroxyl radical, and also by protecting linoleic acid from oxidation. In addition, the flaxseed peptide mixture showed potential antihypertensive property by up to 70.8% inhibition of angiotensin converting enzyme (ACE) activity; 50% inhibitory concentration was 0.16 mg/ml. Kinetics studies showed that the peptide mixture inhibited ACE in a mixed-type inhibition pattern. Protein hydrolysates with Fischer ratio >20 and Phe/Tyr content <2% have been used to restore plasma amino acid imbalance during treatment of patients with hepatic encephalopathy. Thus, this multifunctional flaxseed peptide mixture could be used to formulate food products with multiple human health benefits during liver diseases, oxidative stress and hypertension.

Identification and Characterization of Sphingosine-binding Protein. Zakir Hossain[1,2], Taro Masuda[3], Osamu Nishimura[1], Ei-ichi Matsuo[4], Tsuyoshi Tsuduki[5], Tatsuya Sugawara[1], Takashi Hirata[1], [1]Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh, [3]Division of Agronomy and Horticultural Science,Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [4]Division of Disease Proteomics, Institute for Protein Research, Osaka University, Osaka, Japan, [5]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

Sphingolipids are highly bioactive compounds that participate in the regulation of cell growth, differentiation, diverse cell functions, and apoptosis. They are present in both plant and animal foods in appreciable amounts, but little is known about their absoption mechanism in human intestine. The Caco-2 cells were cultured up to 14-21 days for differentiation. A 40 μM sphingosine with FBS free medium was added and incubated for 2 h. Sphingsine bases treated cells were lysed and sample was loaded to the DEAE column at a flow rate 1ml min^{-1}. Fractions were analysed on fluorescence detector HPLC system. Sphingosine was detected on a TSK gel ODS-80Ts QA (Tosoh), 4.6 x 250 mm, attached to a precolumn (2 x 20 mm) of Pelliguard LC-18 (Supelco, Bellefonte, PA). Sphingosine was identified comparing the peak of a standard solution. Identified fraction was put into a 3-kDa cut-off centrifugal filter and centrifuged at 13000 rpm for 20 min until around 80% of the fluid had passed through the filter. SDS-PAGE was carried out using 15% acrylamide gels. Bands were excised from the gels. Protein pieces were washed sequentially with acetonitrile and ammonium bicarbonate. Proteins were reduced by treatment with 100 mM DTT for 30 min, and

alkylated with 100 mM indoacetoamide. The digestion was accomplished with 25 µg/ml trypsin at 37°C overnight. Tryptic peptides were analyzed by MALDI-TOF. The major peaks obtained by MALDI-TOF were selected to be further characterized by TOF/TOF analyses. Spectra were submitted for database searching in a generic MASCOT format. The identified sphingosine binding protein is PDIA3. These results suggest that absorption of bioactive sphingosine takes place in the human intestine through binding with PDIA3.

Effects of Extrusion Conditions on the Molecular Structures and Functional Properties of WPI. P. Qi, C. Onwulata, USDA, ARS, ERRC, USA

It has been demonstrated previously that snack food products containing extruded whey protein isolate (WPI) possess beneficial nutritional properties and desirable texture, one of many important functional properties. The effects of extrusion conditions such as temperature and moisture content on the molecular structures and functional properties of WPI, however, remain poorly understood. In this work, we studied the effects of the two most important parameters, moisture and temperature, on the molecular structures of extruded WPI using atomic force microscopy (AFM) and scanning electron microscopy (SEM), two complementary microscopic techniques. The high resolution AFM images showed a growing degree of large uniform network of protein aggregates caused by extrusion compared to the randomly scattered aggregates found in the non-extruded samples. Extrusion temperature induced greater changes in molecular structure than moisture as seen by AFM and SEM in this work, consistent with previous spectroscopic findings. The formation of intermolecular disulfide bonds in b-lactoglobulin, speculated previously, may account for the relatively uniform protein aggregates, and may also contribute to the improved functionality of foods containing extruded WPI.

Reducing Hemoagglutination Activity of Soy Proteins. Y. Ma, R. Faris, T. Wang, M. Spurlock, Iowa State University, USA

A series of experiments was conducted to reduce the hemagglutination activity of the anti-nutritional factor in soybeans, i.e. soybean agglutinin (SBA). Deglycosylation decreased its activity, but not as much as heat denaturation. Individual proteases can?t hydrolyze native SBA, but hydrolyzed denatured SBA. However, even after hydrolysis, SBA?s activity still wasn?t fully eliminated. Combination of enzymes with thermolysin fully deactivated SBA and β-conglycinin's activity but not that of glycinin. Hydrolysis by pepsin and pancreatin fully deactivated SBA, but not for β-conglycinin and glycinin, and this procedure doesn?t involve excessive heating. Therefore, it was chosen for treating soy white flake (SWF) which served as a model for the proteins recovered from non-heating aqueous extraction process. In vitro study showed that the SBA in SWF was deactivated by this treatment, but not β-conglycinin and glycinin. In vivo feeding trial didn?t show improvement on chick growth performance compared to the raw SWF, but the chicks didn't show any pancreas enlargement or intestine weight increase, indicating the deactivation of anti-nutritional factors in the material. Finally, two peptides from SBA, three peptides from β-conglycinin were identified to have hemagglutination activity.

Ovotransferrin-derived Peptides Inhibits TNFα induced Inflammatory Response in Endothelial Cells. K. Majumder[1], W. Huang[1], S. Chakrabarti[2], S. Davidge[2], J. Wu[1], [1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, [2]Department of Obstetrics & Gynecology and, University of Alberta, Edmonton, Alberta, Canada

Ovotransferrin consists about 11% of total egg white protein. Three potent angiotensin converting enzyme (ACE) inhibitory peptides (IRW, IQW and LKP) were identified in ovotransferrin. Since vascular inflammation has a major role in hypertension as well as in atherosclerosis, the purpose of the study is to investigate the structure and anti-inflammatory activity relationship of the peptides (IRW, IQW and LKP) in human umbilical vein endothelial cells (HUVECs). Results exhibit that TNFα significantly increased the expression of the inflammatory factors, ICAM-1, VCAM-1 and MCP-1 in HUVECs. The peptide IRW significantly reduced TNFα induced vascular inflammation. It inhibits the MCP-1 production and levels of the endothelial adhesion molecules (ICAM-1, VCAM-1) in a concentration-dependent manner. On the other hand, another peptide IQW showed a modest decrease in TNFα induced vascular inflammation. It inhibits the production of the endothelium adhesion molecules (ICAM-1 and VCAM-1) but not as significant as IRW. Our results demonstrated the efficacy of egg derived bioactive peptides in inhibiting the

inflammatory response of endothelial cells; our results also indicate that the structure of peptides plays an important role in determining the anti-inflammatory activity.

Production of Bioactive Peptides from Spent Hens. W. Yu, C. Field, J. Wu, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

Spent hens are seen as a by-product of the egg industry and of little economic value to the farmers. Traditionally they were used to make chicken soup stock with the meat incorporated. However, this use has been declined because of the insufficient revenue generated from the processing and an objectionable toughness due to its high amount of heat-stable collagen. Alternatively, spent hens have been processed in feedstuffs for animal diets but there are increasing concerns over the safety of using animal byproduct ingredients in animal diets. Finding methods of utilization, other than disposal or conventional food and feed uses, is of great interest. We will study the feasibility of production of bioactive peptides from protein-rich spent hens. Meat proteins will be extracted first according to established methods and several enzymes will be studied to determine their efficiencies in releasing bioactive peptides. To the best of our knowledge, there is no study on exploring bioactive peptides from underutilized spent hens. It is expected that a method will be established to produce peptides with certain functional properties from underutilized spent hens, which will significantly benefit the egg industry by generating additional revenue instead of a liability, improving animal welfare, being environment-responsible, and remaining sustainable.

Processing Method Used to Crush *Camelina sativa* Inhibits Myrosinase Activity in Camelina Meal. Cameron Murphy[1,2], Eric Murphy[1,2], [1]Agragen, LLC, Cincinnati, OH, USA, [2]University of North Dakota, Grand Forks, ND, USA

Myrosinase is an S-glycosidase that catalyzes the hydrolysis of glucosinolates producing glucose, isothiocyanates, and nitriles. Because isothiocyanate blocks the Na/I symporter in the thyroid gland causing goiter, limiting enzyme activity during seed processing is important for downstream use of the meal. Cytosolic myrosinase activity was determined in raw seed, conventionally milled seed, and extruded seed of Camelina sativa using p-nitrophenyl-β-glucopyranoside (NPG, λ_{max}=402nm) as a substrate. Because Camelina cytosol strongly absorbs from 200-400nm, the cytosol was mixed with activated charcoal to reduce its absorbance to an acceptable limit, and the charcoal was removed by ultracentrifugation (131,000 xg for 1h at 4°C). Despite reducing the cytosolic absorbance, sinigrin (λ_{max}=227nm), the conventional substrate, could not be used. Enzyme activity was determined in duplicate using 250µg and 500µg total protein as determined by conventional Bradford assay using BSA as a reference. Myrosinase activity in extruded meal was reduced 91% compared to whole seed, whereas only a 39% reduction in activity was found in conventionally milled meal versus seed. Thus, there is a significant reduction of myrosinase activity using the extrusion processing method, enhancing the value of Camelina meal as a viable source for livestock nutrition.

Protein and Co-Products Posters

Chair(s): P. Qi, USDA, ARS, ERRC, USA; and N. Deak, Solae Co., USA

Antioxidant and Renin Inhibitory Properties of Hempseed (*Cannabis sativa* L.) Protein Hydrolysate Fractions Evaluated in vitro.

A.T. Girgih[1], R.E. Aluko[1,2], [1]Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada, [2]The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada

Food protein-derived bioactive peptides have been used as therapeutic tools for the management of human chronic disease conditions. This study investigated the in vitro antioxidant and renin inhibitory properties of hempseed protein hydrolysate (HPH) and its ultrafiltration fractions. Hempseed protein isolate was hydrolyzed using pepsin followed by pancreatin to mimic the human gastrointestinal digestion. The HPH was fractionated by ultrafiltration using

sequentially, 1, 3, 5 and 10 kDa molecular-weight cut-off membranes. The peptide fractions showed antioxidant property by scavenging 2,2-diphenyl-1-picrylhydrazyl radical, chelating metal ion and strongly inhibiting linoleic acid oxidation. The peptide fractions exhibited weak ferric reducing antioxidant power when compared to glutathione. The antioxidant property of these peptides was dependent on their molecular sizes. In addition, hempseed peptide fractions of 3 and 5 kDa sizes displayed potential antihypertensive property by inhibiting human renin activity. The results indicate that these peptide fractions possess the potential to be used for the treatment of oxidative stress-related diseases and hypertension, thereby contributing to the value-added use of hempseed for the formulation of functional foods and nutraceuticals.

Rapeseed Protein Applications: Aqua Feeds.

H. Adem[1], F. Pudel[2], R.-P. Tressel[2], H. Slawski[1], C. Schulz[1], [1]GMA - Gesellschaft für Marine Aquakultur GmbH, Büsum, German, [2]Pilot Pflanzenöltechnologie Magdeburg, Magdeburg, Germany

Aquaculture is rapidly growing worldwide. The main problem for its sustainable development is the supply of sufficient fish fodder of high quality. As a result of an increasing shortage and price for fish meal proteins particularly alternative protein sources are needed which ensure both cost effectiveness and physiological quality. The by-products of rapeseed oil production, cake or meal, represent a low cost protein source.Therefore the suitability of rapeseed proteins for fish feeding was investigated. In small pilot scale different rapeseed protein products were produced: concentrates (> 60 % protein) and isolates (> 90 % protein) as well as globulin and albumin rich fractions. By variation of the processing parameters the contents of antinutritive substances, like glucosinolates, phytic and sinapic acid, and the process efficiency were adjusted. The protein products were fed to rainbow trouts (*Oncorhinchus mykiss*) and turbots (*Psetta maxima*). The presentation gives the first results.

Purification and Characterization of Gli-B1: A Major Allergen of Wheat-dependent Exercise-induced Anaphylaxis.

Y. Nakayama[1], M. Tanaka[1], T. Nezu[1], Y. Kato[1], T. Ikeda[2], T. Nagano[1], [1]Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan, [2]Agricultural Research Center for Western Region, Fukuyama, Hiroshima, Japan

Gli-B1 and Gli-D1 are the same ω-gliadin components. Gli-B1 is reported as a major allergen of wheat-dependent exercise-induced anaphylaxis (WDEIA) but Gli-D1 is not. In this study, we purified Gli-B1 and Gli-D1, and examined antibody responses in mice. Gliadin was extracted from soft wheat flour with 70% (v/v) ethanol. Gli-B1 and Gli-D1 were then purified using ion-exchange chromatography and size exclusion chromatography. SDS-PAGE showed that the purified Gli-B1 and Gli-D1 are three bands around 65-55 kDa and a single band around 50 kDa, respectively. The N-terminal amino acid sequences of these protein bands in SDS-PAGE were identical to Gli-B1 or Gli-D1. We examined the antibody responses provoked in mice by Gli-B1 and Gli-D1. B10.A strain mice were sensitized by interperitoneal injection with gliadin/alum. The IgE and IgG1 binding ability to the purified Gli-B1 or Gli-D1 was studied by enzyme-linked immunoadsorbant assay (ELISA) using the serum of the gliadin-sensitized mice. The sensitivities of the IgE and IgG1 were high for Gli-B1 and low for Gli-D1. In conclusion, Gli-B1 provokes IgE and IgG antibody response but Gli-D1 has a little impact on antibody responses in mice.

Impact of Processing Conditions on the Color and Flavor of Canola Protein Extracts.

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In this work, we investigated the impact of modifying processing conditions on the color, flavor and physico-chemical properties of canola protein extracts. The process used in the study consisted of three main steps: extraction, precipitation, and ultrafiltration/diafiltration. Three protein products were prepared: canola insoluble protein (CIP), canola soluble protein (CSP) and protein recovered in the ultrafiltration permeate (CSP-P-UF). Protein recoveries and purities varied from 0.3 to 27% and 12% to 95%, respectively. Gel electrophoresis revealed the presence of proteins varying in MW ranging from 14 to 50 kDa. Protein profiles of the extracts were affected by the processing conditions used. Color and flavour of the CIP, CSP and CSP-P-UF were also significantly affected by changes in extraction conditions. Our result clearly showed that modifications in processing conditions could help improve the color and flavour of canola protein products which could help enhance their potential use as value-added products in food

application.

Monoglycerides and Diglycerides Preparation Using Glycerin, Co-products of Biodiesel Industry.
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Glycerin production has soared with the increase in biodiesel production in recent years. One possibility of using this excess glycerin is in monoglycerides (MAG) and diglycerides (DAG) production. Mono and di- esterified glycerols were synthesized by two different methods: using p-toluenesulfonic acid and with enzyme (immobilized lipase from Candida antarctica Novozym 435) as catalyst for reactions of glycerol with oleic acid in a solvent-free system, the water generated by esterification was removed by vacuo. The reaction was carried out at a experimental design with glycerol to oleic acid molar ratio of 3:1 at 8:1 with 0.5 to 1.5% of lipase at 50 to 70ºC; and The experimental range was from 1.0 to 2.5% of p-toluenesulfonic acid at 110 to 150°C. High-performance size exclusion chromatography (HPSEC) was used to evaluate triglycerides (TAG), DAG, MAG and free fatty acids (FFA). The operating conditions that optimized MAG and DAG yields was the molar ratio of 8:1 for glycerol:oleic acid in both methods. The use of 2.5% p-toluenesulfonic acid at 150ºC, leading to a content of 38 and 53% of MAG and DAG, respectively. With 1.5% of Novozym 435 at 50ºC the MAG and DAG yields were 27 and 53%, respectively.

Physicochemical and Functional Properties of Defatted Canola Meal Residue and its Potential Use as a Source of Dietary Fibre.
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In this paper, we studied the physical, chemical and functional properties of defatted canola meal residue and compared the results with those reported for other fibre products in the literature. Canola press cake was defatted with hexane and protein was extracted twice using an alkaline solution (pH > 10) and the residue was dried to obtain the canola meal residue. Physico-chemical analysis of the meal residue revealed fibre and protein contents of 66.87±0.87% and 22.24±1.8%, respectively. The meal residue had a characteristic greenish-yellowish colour with L, a, b values of 66.03 ±1.92, -2.13 ±0.38 and 10.92 ±0.91, respectively. Bulk density was 300 kg.m-3, and fat absorption and water holding capacities were