2010 Annual Meeting Abstracts

Phospholipid

MONDAY

MORNING
PHO 1: Nutritional and Biological Functions of Polar Lipids
Chair(s): G. Wang, Iowa State University, USA; and X. Xu, University of Aarhus, Denmark

****CANCELED** Effects of Different Phospholipids on Gastric- and Pancreatic-lipase Activity and Establishment of the Gut Microbiota.** L.I. Hellgren

Effect of Adding Milk Fat Globular Membrane (MFGM) to Anhydrous Milk Fat on Lipid Metabolomics in Fischer 344 Rats Compared to Corn Oil Control. Robert E. Ward, Korry Hintze, Albert Zhou, Dallin Snow, Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT 84341, USA

Milk fat has a unique native structure wherein the fat globules are surrounded by a bilayer membrane which originates from the apical surface of the lactating epithelial cell. In the production of butter from cream, the milk fat globule membrane (MFGM) is separated from the butter solids, and is recoverable from the resulting buttermilk for use as a potential nutraceutical. Despite its unique composition, few in vivo studies have been conducted with MFGM to determine biological effects. We recently fed Fisher 344 rats the AIN-76A diet with the fat source as a) corn oil b) anhydrous milk fat (AMF) or c) anhydrous milk fat with additional mfgm. To understand the effects of the supplementary MFGM we collected plasma, adipose, skeletal muscle and liver tissues for gene expression and lipid metabolomics analysis. As expected, the corn oil diet led to high levels of omega-6 fatty acids in tissues compared to the milk fat based diets. However, distinct differences were noted between the milk fat based diets. For example, addition of MFGM led to higher concentrations of plasma phospholipids and triglycerides in plasma, compared to AMF, and in liver this was reversed. These data suggest that addition of dietary phospholipids significantly affect lipid partitioning and transport.

Effect of Milk Sphingolipids on Rodent Plasma and Liver Lipids. S. Watanabe[1], T. Takahashi[1], L. Tanaka[2], Y. Haruta[2], M. Shiota[2], M. Hosokawa[1], K. Miyashita[1], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Japan, [2]Snow Brand Company, Kawagoe, Japan

Major component of milk lipids is triacylglycerols (95 to 98%). Main fatty acids of milk triacylglycerols is saturated fatty acids (SFA; approximately 70%), while the amount of polyunsaturated fatty acids is less than 3%. On the other hand, CLA found in milk lipids has been considered to protect against CHD. Milk lipids contain polar lipids which may be expected to show health benefit effect. In this presentation, the effect of milk shingolipids on liver and plasma lipids is reported. Milk phospholipids including sphingoglycolipids and shingomyelin were obtained from waste matters (butter serum) after production of cream and butter. Butter serum is a good source of these shingolipids, mainly composing of glucosylceramide, lactosylceramide, and shingomyelin. Feeding of milk sphingolipids to Wister rats significantly reduced the plasma cholesterol levels and liver triacylglycerols as compared with the control rats. The same result was also obtained in diabetes/obese model mice, in which the fraction of milk ceramide showed the highest activity as compared with other polar milk lipid fraction.

Analysis of Possible Pathways Involved in Buttermilk's Antiproliferative Activity in in vitro Studies. A. Kuchta[1,4], R. Devery[1,2], B. Murray[3], C. Stanton[4], P. Kelly[3], [1]School of Biotechnology, Dublin City University, Dublin, Ireland, [2]National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland, [3]Department of Food Processing and Functionality, Moorepark Food Research Centre, Fermoy, Ireland, [4]Biotechnology Centre,

Moorepark Food Research Centre, Fermoy, Ireland

Buttermilk is naturally rich in milk fat globule membrane (MFGM) polar lipids and proteins with defined anticancer properties. We previously reported that buttermilk possessed antiproliferative activity on SW480 human colon cancer cells and had no effect on FHC human normal colon mucosa cells. The aim of this study was to determine the biochemical pathway responsible for buttermilk's antiproliferative activity. Analysis of cell cycle, mitochondrial permeability and phoshatidylserine exposure in cells using flow cytometry suggest induction of apoptosis after 24 h of buttermilk treatment at the concentrations as low as 1 g total solids/L. Further studies showed that sugar source (glucose versus galactose) in cells' feeding media might possibly effect buttermilk's antiproliferative activity as shown in acid phosphatase activity assay. Mode of action on colon cancer cells of buttermilk's components is not fully understood and needs to be studied further.

AFTERNOON

PHO 2 / EAT 2.1: Lipids in Nanotechnology I

Chair(s): S. Ali, Jina Pharmaceuticals, Inc., USA; M. Ahmad, Jina Pharmaceuticals, Inc., USA; and F. Orthoefer, FTO Food Research, USA

Micro/nanochannel Emulsification for Producing Monodisperse Emulsions Containing Lipids. I. Kobayashi[1], M. Nakajima[2,1], [1]National Food Research Institute, NARO, Tsukuba, Ibaraki, Japan, [2]University of Tsukuba, Tsukuba, Ibaraki, Japan

Monodisperse emulsions consisting of uniform droplets are dispersions useful for foods, pharmaceuticals, cosmetics, chemicals, etc. Microchannel (MC) emulsification, proposed by our group in the middle 1990s, is a promising technique to produce monodisperse emulsions with the smallest coefficient of variation of below 5%. Droplet generation for MC emulsification is very mild and does not require any external shear stress. Here we introduce recent developments in MC emulsification using lipids as an emulsion component. Initially designed MC emulsification chips composed of silicon grooved MC arrays with microgrooves and a terrace. Straight-through MC arrays with straight through-holes have been recently developed for large-scale production of monodisperse emulsions. MC emulsification can generate uniform droplets of refined soybean oil (RSO) with an average size of 2–200 μm, dispersed in a continuous aqueous phase. Straight-through MC arrays demonstrated a high productivity of uniform RSO droplets of 100 L m-2 h-1. Monodisperse oil-in-water and water-in-oil emulsions stabilized by phospholipids have been produced by MC emulsification. NC emulsification for producing submicron emulsions is also briefly introduced. This work was supported in part by the Food Nanotechnology project of MAFF, Japan.

Structured Lipids in Physical Structuring: Case Studies. Xuebing Xu, Aarhus University, Aarhus C, Denmark

We have knowledge that structured lipids, after chemical re-structuring, have better bioavailability or better delivery to specific parts of the body. The case for example for the structure containing omega-3 PUFA at the sn-2 position and medium chain fatty acids at the sn-1,3 positions has accumulated quite amount of evidence. When structured lipids are made in specific emulsion for animal model study, particularly in different emulsion structures, the delivery of omega-3 PUFA to the brain was found significantly different. This is some cases can improve 20-30% of bioavailability. A couple of other cases to use phospholipids as carrier of omega-3 PUFA for liposome formulation also showed the difference of bioavailability. This indicates the physical structure of bionutrients in intaking can give a different performance.

Milk Phospholipids Nanoliposome as Bioactive Compounds Carrier. B. Farhang, Y. Kakuda, M. Corredig, University of Guelph, Guelph, ON, Canada

Nano liposome technology has been considered as an effective technology in food industry for encapsulation and controlled release of nutraceuticals and bioactive compounds, as well as for enhancing their stability and bioavailability. Liposomes are mostly prepared with soy or egg phospholipids. This research focuses on the study of nano liposomes prepared from milk phospholipids. These have been shown to have significantly different physical and

chemical characteristics than those traditionally prepared with soy . We have prepared different types of milk liposomes via microfluidization. These nano liposomes would themselves be considered bioactive ingredients, because of the high content of Sphingomyelin present in the milk phospholipids. To characterize the encapsulating behaviour of the liposomes, we will use model hydrophobic and hydrophilic compounds, namely β-Carotene and Vitamin C. Preliminary results showed high incorporation efficiency for β-Carotene and a significant increase in the stability of vitamin C. These results suggest that there may be inherent advantages in the use of nano liposomes prepared from MFGM-derived phospholipids via microfluidization for the encapsulation of both hydrophobic and hydrophilic compounds, in addition to the nutritional benefits of the milk phospholipids per se.

Lecithin-based Self-Emulsifying Oral Delivery Systems. J. Chu, E. Acosta, University of Toronto, Toronto, ON, Canada

Lipid-based formulations have been used to improve the oral bioavailability of hydrophobic drugs. The lipid-based, self-emulsifying drug delivery system (SEDDS) shows potential in improving oral bioavailability of poorly water soluble drugs. We developed alcohol-free SEDDS formulated with lecithin, linkers, and food-grade additives. The linker system is comprised of sorbitan monooleate, a lipophilic linker, decaglyceryl caprylate/caprate and PEG-6-caprylic/capric glycerides which are hydrophilic linkers. Ethyl caprate was the carrier oil for lipophilic nutraceuticals beta-sitosterol and beta-carotene. A ternary phase diagram was generated to investigate the phase behaviour. Mixtures of surfactants/ linker in aqueous medium and surfactants/ linker in oil medium were titrated with oil and aqueous components respectively to determine the dilution lines, isotropic regions, and phase transitions of microemulsion structures. Microemulsion preconcentrates were formulated which could be incorporated into pharmaceutical or other applications. The diluted preconcentrates form self-emusified drug delivery systems with drop sizes ranging from 100-250 nm. The equilibrium uptake and permeation of the oil phase (containing the drug) through a membrane was evaluated using a Flow-Thru Dialyzer system.

Phenolipids: Novel Phenolics Enriched Lecithin for Functional and Pharmaceutical Applications. M.F.R. Hassanien, Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Egypt.

Phenolipids results from the reaction of phospholipids with the selected phenolics in a nonpolar solvent. They are lipophillic substances freely soluble in nonpolar solvents (in which the hydrophilic moiety was not), and moderately soluble in fats. Liposomes, unlike Phenolipids, are formed by mixing water-soluble substances with phospholipids without forming chemical bonds. This difference results in Phenolipids being much better absorbed than liposomes or individual phenolic compounds. Preparation of Phenolipids is recently described by complexing querectin with soy lecithin [1]. Phenolipids exhibited novel antioxidant properties in a triolein model system stronger than individual lecithin or querectin. The goal of this work was to optimize preparation of different structured Phenolipids and to study antioxidant properties of new Phenolipids. Phenolipids are anticipated to play a vital role in efficient herbal drug delivery of a broad spectrum of protective phytochemicals. After selection of potential phytochemicals from medicinal plants, Phenolipids can be developed for various therapeutic uses like cardiovascular, anti-inflammatory and anticancer activities. Moroever, Phenolipids are anticipated to show their potential in cosmetics as anti-skin ageing agents and for the use of other nonpathogenic skin conditions. [1] Ramadan MF (2008) Food Science and Technology-LWT 41: 581-587

TUESDAY

AFTERNOON

PHO 3: Analytical Characterization and Quantification in Phospholipids

Chair(s): B. Diehl, Spectral Service GmbH, Germany

Milk Lecithin Reference Standard is Available. B.W.K. Diehl, Spectral Service, Germany

Analogue to the mixed soy bean lecithin standards of the I.P.L.S. a reference standard for the analysis of milk lecithin is available. The phospholipid distribution was determined by NMR spectroscopy using a 600 MHz cQNP instrument.

The fatty acid composition of the single components were analysed by HPLC/MS and GC/MS. The standard enables a unique worldwide analysis of milk lecithin with 31P-NMR, HPLC/LSD or other analytical tools.

^{1}H-NMR, an Alternative Method for Analysis of Peroxide Values in Oils and Lecithin. G. Randel[1], K. Oelke[2], [1]Spectral Service, Germany, [2]Christian Albrechts University Kiel, Germany

The strong enhancement of the signal to noise ratio in NMR spectroscopy caused by the cryo technologies enables the quantitative analysis of small amounts of peroxides in fatty oils by direct observation of the peroxide protons. The LOD actually is a POZ of 0.5. The NMR method was cross validated against the Thiocyanate method . In addition to the analysis of the sum parameter NMR distinguishes between the species of peroxidation in different fatty acids.

Qualitative and Quantitative Analysis of Glycolipids and Neutral Lipids in Soy Lecithin. B.W.K. Diehl, Spectral Service, Germany

The combination of SPE and NMR spectroscopy is used to obtain the amounts of glycolipids (MGDG, DGDG, sterylglycosides, sterylglycosidesters and cerebrosides). From acetone soluble parts of soy lecithin the amounts of neutral Lipids (tri- di- and monoglycerides, free fatty acids and free sterols) were determined. Validation and results of routine analysis were presented and compared with LC/MS data.

PHO 3.1: Lipids in Nanotechnology II
Chair(s): S. Ali, Jina Pharmaceuticals, Inc., USA, and M. Ahmad, Jina Pharmaceuticals, Inc., USA

Novel Architectures Based on Lipids and Oil Derivatives as Delivery Vehicles. Nissim Garti, Casali Institute of Applied Chemistry; The Hebrew University of Jerusalem, Jerusalem, Israel

Fats are considered unhealthy and products rich in saturated oils (fats) are less attractive to the sophisticated consumers. Efforts are made by scientists, technologists in academia, institutes and industry, to find novel oil-based structures as fat-replacers. Scientists are constantly considering new possible options for ?structuring vegetable oils? by lecithins and other polar lipids to reduce the fats content in the lipidic matrix of foods. Other scientiscts are attempting to form ?structured mesophases into oils?. These novel architectures must mimic the fats and be efficient carriers for oils, additives and bioactives. Several new and very brilliant concepts and innovative ideas have been suggested in recent years to fulfill these functions. Some ideas are based on controlled crystallization of small amounts of fats that are incorporated into liquid oil matrices. Another concept tends to nucleate waxes, monoglycerides, derivatized fatty acids into oils. Others are dispersing polymers into oils to form viscoelastic networks with fibrils in the oils. We considered another option which is not structuring the oils but forming semisolid (gelled) lipid architectured mesophases such as gelled Lyotropic Liquid Crystals (LLC) constructed with phospholipids and monoglycerides, Oleotropic Liquid Crystals (OLC) from mixed lipid-based ingredients.In this presentation a critical review of the proposed structures formed in oils, methods to structure the oils and methods to form new mesophases into oil-based systems will be discussed. All the systems have some potential and some limitations as fat replacers and/or as vehicles for bioactives into fat based systems.In particular we will discuss lyotropic liquid crystals such as lamellar, reverse hexagonal, cubic, sponge and ribbon mesophases and the corresponding soft dispersed particles.The bioavailability and reactivity of addend that are solubilized in these new structures will also be demonstrated.

Designed Phospholipid Self-assemblies in Drug Delivery and Nanomedicine. Heidi Mansour, University of Kentucky, College of Pharmacy, Lexington, KY, USA

This is a 20-minute invited oral presentation for the technical session "Lipids in Nanotechnology" with a focus on pharmaceutical drug delivery applications in nanomedicine. Various aspects of our research using phospholipid and lipopolymeric self-assemblies in pharmaceutical applications will be presented. These nanomedicine applications include pharmaceutical formulations and drug delivery as liposomes and polymeric liposomes in the colloidal dispersion state and in the solid-state. The surface chemistry, biophysics of biomembranes, material science, and solid-state vitrification properties that influence the performance of these nanomedicine drug delivery systems will also be discussed.

Investigation on the Influence of Well-defined Cooling Rates on the Crystallization and Polymorphism of Triglyceride Nanoparticles using a Microfluidic Approach. S. Fehr[1], V. Huzhalska[2], W. Augustin[2], S. Scholl[2], H. Bunjes[1], [1]Institut für Pharmazeutische Technologie, Technische Universität Carolo-Wilhelmina zu Braunschweig, Mendelssohnstr. 1, D-38106 Braunschweig, Germany, [2]Institut für Chemische und Thermische Verfahrenstechnik, Technische Universität Carolo-Wilhelmina zu Braunschweig, Langer Kamp 7, D-38106 Braunschweig, Germany

Solid lipid nanoparticles are investigated as drug carriers and may also find applications in food technology. They are usually prepared by high-pressure melt homogenization of a matrix lipid in a surfactant-containing aqueous medium and subsequent cooling to crystallize the resulting lipid nanodroplets. The cooling rates are typically low and often poorly defined. The use of high, well-defined cooling rates might offer new possibilities for the manufacturing of these nanoparticles. In this study, continuous melt crystallization of tristearin nanoparticles was performed in a micro heat exchanger at very rapid cooling rates in comparison to batch-wise cooling in a thermostat. Further points of interest were the influence of the matrix lipid concentration, the emulsifier (poly(vinyl alcohol), sodium glycocholate alone or combined with hydrogenated soybean lecithin) and the storage temperature. The crystallized nanoparticles were analyzed after 0, 4 and 12 weeks of storage with regard to particle size, suspension stability and effects on the polymorphic transformation of the triglyceride. The type of emulsifier and the storage temperature showed a more pronounced influence on tristearin polymorphism than the cooling rate.

WEDNESDAY

MORNING
PHO 4: Non-Food Applications and General Phospholipids
Chair(s): B. Sebree, Archer Daniels Midland Co., USA; and K. Seabolt, Solae Co., USA

Novel Bio-based Pigment Dispersants for Coating Applications. S. Baseeth, D. Salyers, B. Sebree, Archer Daniels Midland Co., Decatur, IL, USA

Pigments impart color to both alkyd and latex paints and coatings. They also can contribute to the opacity, durability and hardness of paint coatings. New environmental regulations, and consumer demand, have led to the development of low-VOC and zero-VOC paints and finishes. Most paint manufacturers now produce one or more non-VOC variety of paint. So using a no-VOC pigment concentrate that has a bioderived dispersant can be of huge advantage as they contribute zero-VOC to the final paint. Bio-derived materials offer an attractive alternative for industrial manufacturers looking to reduce or replace their reliance on petrochemicals and petroleum derived products. The disclosed water dispersible lecithin-based compositions find utility in water-based coatings, including, but not just limited to the latex paints. Also, the dispersants were applied as vehicles for pigments in paint and ink formulations. The low viscosity offered by this dispersant provided improved coating uniformity to pigments and other particulates in dispersions. The unique dispersant, wetting agent, and/or stabilizer properties and performance by exhibiting low-grind viscosity, high pigment load, low foam, high color development, and fast dispersion/wetting in a architectural coating will be discussed.

Functionality and Uses of Lecithin in Feed and Pet Foods. Bruce Sebree, Archer Daniels Midland Co., Decatur, IL, USA

An overview of the use and benefits of lecithin and lecithin products for the feed and pet food industries will be presented. Nutritional aspects for various species, as well as technical function in processing will be discussed. Soybean lecithin consists mainly of phospholipids. Phospholipids are ?polar lipids?, which are important substances for the structure of all biological processes, and are particularly essential for lipid metabolism. The phospholipids in lecithin are important in promoting fat transport between the liver and tissue, and in metabolite exchange between cells, since the functional constituents of cell membranes consist mainly of phospholipids. Lecithin is commonly used as an emulsifier to improve the digestion, absorption, transportation and utilization of nutrients, as a production aid in food and animal feed - helping the formation of water in oil or oil in water emulsions, dispersion of particulates, dust

reduction and lubricity. Lecithin?s emulsifying properties enhance the effect of digestive enzymes on the substrate nutrients and influence the digestion and absorption of nutrients. Fats are insoluble in water and difficult to handle in aqueous media such as in the digestive tract. Lecithin can increase fat dispersion in the digestive tract of animals and therefore improve fat utilization. Emulsified fats tend to improve overall feed absorption by slowing the passage of feed through the digestive tract. In extrusion types of diet, lecithin can provide the necessary lubrication, allowing more efficient feed production and improved pellet integrity and stability. In both fluid and granular forms, lecithin helps bind pellet feed, increases the throughput of mechanical pelleting equipment and reduces the leaching of water-soluble nutrients.

Overview of Lecithin Uses and Functionality for Industrial Products. K. Allen Seabolt, Solae, St. Louis, MO, USA

A wide range of commercial lecithin products exist which contribute release, emulsification, instantizing and other benefits to industrial, non-food applications. Lecithin also acts as a dispersing agent, wetting agent, penetrating agent, mixing aid, viscosity modifier, antifoam and emollient for industrial applications. It can be specially produced to have a low viscosity for spray applications and it can be modified to improve water dispersibility. The use of lecithin improves the release of plastic, concrete, asphalt and other substances where sticking to molds or processing surfaces is a concern. It improves the surface quality of molded products, reduces product loss and improves production time. It can be used as an emulsifier for products such as paints, cosmetics and medications. In paints it helps with pigment dispersion, wax dispersion and scuff resistance. Powders that are difficult to wet and disperse such as waxes can be instantized by applying lecithin to the surface. Lecithin is used in textiles and leather, coatings, ink, paper, plastic, and metal processing fluids. In cosmetics the addition of lecithin softens and smooths the skin. Its phosphatidylcholine content and ability to form liposomes make it an especially functional component for skin care and pharmaceutical products. Environmental applications for lecithin include bioremediation and earth-friendly agricultural chemicals.

Glycerol Phosphocholine and Phenethyl Alcohol Extraction from Thin Stillage. Kornsulee Ratanapariyanuch[1], Yunhua Jia[2], Jiangheng Shen[1], Martin Reaney[1], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N5A8, [2]College of Medicine, University of Saskatchewan, A202 Health Sciences Building, 107 Wiggins Road, Saskatoon, Saskatchewan, Canada, S7N5E5

Glycerol phosphocholine (GPC) has been used in several applications such as, drug in the treatment of cognitive disorders specific to adults, including Alzheimer's disease. Phenethyl alcohol (PEA) has been utilized in fragrances and it has antimicrobial properties. Since GPC and PEA are the expensive compounds, they should be recovered from potential sources. Here we focused on recovering GPC and PEA from thin stillage (TS), liquid waste, obtained from Pound-Maker Agventures Ltd. Analysis of organic compounds present in TS was conducted using water suppression proton NMR. Isopropanol, ethanol, lactic acid, 1,3 propanediol, acetic acid, succinic acid, GPC, betaine, glycerol, and PEA, yeast, bacteria and grain metabolite, were the organic constituents in TS that were able to be identified. For GPC extraction, TS was evaporated to obtain TS syrup which was diluted with acetone to extract non GPC soluble compounds. After acetone extraction, methanol was then added to the mixture to extract GPC, betaine and lactic acid. PEA may be recovered from TS using solvent-solvent extraction. Two methods were used to extract PEA from distilled TS: 1) dichloromethane and 2) canola oil and isopropyl alcohol extraction respectively. Hence, TS could be a potential source to produce enough GPC and PEA for supplying to the industries.

Effect of Processing Conditions on Enzymatic Hydrolysis of Sunflower Lecithins. D.M. Cabezas[1], R. Madoery[2], B. Diehl[3], M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (FCE UNLP CONICET), La Plata, Bs As, Argentina, [2]Fac. de Cs. Agrarias (UNC), Córdoba, Argentina, [3]Spectral Service, Cologne, Germany

Lecithin is a multifunctional ingredient used in a wide range of industrial applications (nutritional, pharmaceutical, cosmetics). Enzymatic hydrolysis can originate lysolecithins which are applied to different food purposes (emulsions, bakery products) due to the interesting functional properties of lysophospholipids (LPL). The aim of this work was to evaluate the enzymatic hydrolysis of sunflower lecithins studying the effect of different operative conditions on the process. Enzymatic hydrolysis of sunflower lecithin was carried out with a PLA2 (LysoMax) (0.4–2.0 ml/100g lecithin,

pH 7–9, 50°C, 40–300 min, 0.4 M CaCl2) under continuous agitation. Reaction products were deoiled and dried under vacuum. Phospholipid (PL) composition of samples was determined by 31PNMR and the process followed by total PL (TPL) and LPL/TPL % ratio. The results showed that this modification process allowed to obtain sunflower lysolecithins with LPL content between 2.6–23.8% (starting sunflower lecithin 1%) and a wide range of LPL/TPL% (4.6–57.5%). Lipase concentration and lipase*pH interaction were significant parameters for both TPL (R2 0.928) and LPL/TPL%. Also, time, lipase*time, time*pH presented relevance for LPL/TPL% (R2 0.998). PC was the main PL converted for all conditions.Hydrolyzed sunflower lecithins constitute an interesting alternative as bioemulsifiers to be applied at the food industry.

Enzymatic Modification of Phospholipids in Milk, and the Effect on UHT-milk Processing. J.B. Soe, N.E. Larsen, Danisco A/S, Denmark

Fouling of heat exchangers in the Dairy Industry is a quite severe problem both technically and economically. During thermal treatment of milk, proteins are often denatured and precipitated to form fouling on the heat exchange surfacesThe equipment typically used to realize an indirect heat treatment of the milk is a Plate Heat Exchanger (PHE) or a Tubular Heat Exchanger (THE). Fouling formation causes increased pressure drop over the PHE/THE which again results in reduced working efficiency through the reduction of heat transfer coefficient, and furthermore results in increased time taken away from actual production (down time) in order to clean the surfaces affected.It has been demonstrated through full scale factory trials that it is possible to reduce the amount of fouling in PHE/THE?s through enzymatic treatment of milk with a Lipid Acyltransferase called FoodPro Cleanline. The enzymatic treatment will greatly reduce fouling rates and will also result in an improved emulsion stability of the milk product(s) and furthermore result in up to 80% reduction in the amount of free cholesterol in the resulting milk product. Food-ProTM Cleanline is a food grade enzyme which is active on phospholipids and cholesterol in milk products. The enzyme primarily catalyses the transfer of acyl groups from the sn-2 position of phospholipids to cholesterol in milk. The transferase reaction contributes to the formation of lysophospholipids and cholesterol esters. Essentially, the transferase reaction controls the formation of lysophospholipids without formation of a significant amount of free fatty acids, because the amount of donor substrate phospholipid is lower than the amount of acceptor substrate cholesterol. This will secure the reduced fouling effect in the heat exchanger and still maintain the high organoleptic quality of the enzyme-treated milk.The antifouling effects of the enzyme treatment can theoretically be explained by the fact that the enzyme produces lysophospholipids which are known to be water dispersible and which have a high surface activity. Laboratory experiments have shown that milk treated with Food-ProTM Cleanline has lower surface tension measured against a stainless steel surface than milk without enzyme treatment. The reduction in surface tension indicates that the enzyme reaction results in the creation of surface- and interfacial active components, which play an important role for the protein's ability to form deposit or to settle on the surface of the heat exchanger.

Phospholipids Posters

Chair(s): G. Randel, Spectral Service GmbH, Germany; and B. Diehl, Spectral Service GmbH, Germany

Effect of Different Enzymatic Treatments on Emulsion Properties of Leftover Egg.
Sahar Navidghasemizad, Yeping Xiong, Jonathan M. Curtis, Feral Temelli, Jianping Wu, Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada

IgY extraction from immunized hen egg yolk greatly increases the value of eggs but produces a large amount of leftover egg yolk pellet. The leftover pellet is an excellent source of choline rich phospholipids which can be used in pharmaceuticals and infant formula. Methods for phospholipids extraction usually involve the use of organic solvents. These may carry safety and environmental concerns. Therefore, a "green" technology development which does not rely on organic solvents for extraction is necessary. Egg yolk is a fluid emulsion which phospholipids are mainly trapped on the surface of lipoproteins of the emulsion. The objective of this study was to understand the emulsion properties of leftover egg yolk upon different enzymatic treatments. Six proteases (Protex 7L, Protex 51FP, Protease A, Protease II, Protease M and Protease P) in combination with Lipase AY were chosen to test their effect on emulsion properties of

digested pellet.Results showed the enzymatic treatments could not demulsify pellet, instead a new creamy emulsion rich in oil was formed. LC/MS analyses showed that a major portion of the phospholipids, composed mainly of phosphatidylcholine, are highly accumulated in the creamy phase. Effect of enzymes combination on the emulsion stability of the digested pellets and SDS-PAGE profile of liberated proteins and peptides was also studied.

Sunflower Lecithin Hydrolysis by Phospholipase A1. Effect of Reaction System.
M.L. Goni, D.T. Constenla, A.A. Carelli, PLAPIQUI (UNS-CONICET), Bahía Blanca, Buenos Aires, Argentina

The effect of the reaction system on the sunflower-lecithin enzymatic hydrolysis by free phospholipase A1 was studied. The reaction system consisted on buffer, substrate (67% of phospholipids, 0.5% water and 32.5% oil) and enzyme in a ratio of 4mL:80mg:1μL. The reaction was carried out in a batch reactor, maintaining time and temperature fixed at 1h and 50ºC, respectively. The effect of pH (5 and 8), addition of calcium ions (0 and 0.24mg/mL) and solvent (0 and 240mg/mL) in the reaction medium was considered. The hydrolysis degree (%H) was determined by titration of liberated fatty-acids (FFA), previously extracted from the reaction medium. The FFA profile generated by hydrolysis was determined by capillary gas chromatography using the internal standard method, procedure which involves lipid extraction, clean up and fatty-acid methyl-esters preparation. Results demonstrated than the use of solvent in the reaction medium causes a decrease in the H%, for any of the parameter combinations, presenting values of H% between 1.5% and 14%, compared with a range of H%= 9%-34% obtained by free-solvent reaction. When the pH and the presence of calcium ions are evaluated, the most affecting parameter was the pH. At pH 5, the highest values of H% were obtained (34% and 28% depending on the addition or not of calcium ions, respectively).

Kinetic Study of Sunflower Lecithin Hydrolysis using Phospholipase A1.
M.L. Goñi, D.T. Constenla, A.A. Carelli, PLAPIQUI (UNS-CONICET), Bahía Blanca, Buenos Aires, Argentina

The effect of temperature and time on solvent-free sunflower-lecithin enzymatic hydrolysis by free phospholipase A1 (PLA1) was studied. The reaction was carried out in a batch reactor, at two different temperatures (50ºC and 60ºC). The reaction medium consisted in a mixture of buffer (pH=5), lecithin (67% of phospholipids, 0.5% water and 32.5% oil) and enzyme in a ratio of 4mL:80mg:1μL. An aliquot of this reaction mixture was taken for analysis at different times (1-24h). The liberated fatty-acids were extracted and titrated in order to determine the hydrolysis degree. The free-fatty acid (FFA) profile generated by hydrolysis was determined by capillary gas chromatography using the internal standard method, with a previous FFA extraction, clean up and methyl-esters preparation. The results show a notable difference between the two temperatures considered, even at short reaction times. When the reaction is carried out at 60ºC, the variation of hydrolysis degree (H%) is very low (from 25% to 30% approximately) and the maximum value is achieved at short reaction times (t= 3h). In contrast, a wide range of H% (30% to 60% approximately) was observed at 50°C.The highest hydrolysis degree (60% at 20h) was obtained at this temperature.

Enzymatic Degumming of Sunflower Oil.
D.L. Lamas, L.N. Ceci, D.T. Constenla, G.H. Crapiste, Plapiqui (UNS-CONICET), Bahía Blanca, Buenos Aires, Argentina

Lecitase Ultra (Phospholipase A1, PLA1, from *Thermomyces lanuginosus*) and Maxapal A2 (Phospholipase A2, PLA2 from *Aspergillus niger*) were used to degumming crude sunflower oil. The assays were carried out in batch system with continuous agitation using the following conditions: buffer concentration=2% v/m (0.1 M sodium citrate/sodium hydroxide, pH=5); temperature=50 °C, enzyme concentrations= 100 and 200 U/kg of oil. Aliquots of reaction mixture were sampled at 30, 60, 120, and 180 min after enzyme solution was added, then were heated 30 min at 100 °C to stop the enzymatic reaction and centrifuged 10 min at 5000 rpm to recovery oil and aqueous phases. The oils were analyzed for phosphorous content (AOCS Ca 12-55) and acidity (IUPAC 2.201). Blanks without enzyme were performed to study the degumming induced by buffer solution. The phosphorous content in crude oil was 899 ± 83 mg/kg and was reduced to about 40 mg/kg after 120 min of treatment with buffer. Booth enzymes produced oils with lower levels of phosphorous, being PLA1 the most effective (oils with minor than 5 mg of phosphorous/kg at 100 U/kg, 120 min). Under the same conditions, PLA2 degummed to phosphorous level of 25-30 mg/kg. The increase in enzyme concentration (200 U/kg) produced oils with lower phosphorous level resulting about 15 and 1 mg/kg for PLA2 and PLA1, respectively.

Novel Synthesis of Guggullipid Derivatives as a Drug Delivery System.
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Determination of Lecithin in Vegetable Oils and Vice Versa Determination of Mono-, Di-, and Triglycerides in Lecithin.
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Vegetable oils are containing low amounts of phospholipids, which have a negative influence on the taste of the oil. Therefore, it is a need to remove these phospholipids. However, an analytical method is necessary to check the remaining phospholipid content in native and processed oils. The combination of 1H-NMR and 31P-NMR provides a valid set of analytical approaches to detect phospholipid residues in vegetable oils with a LOD of 10 ppm. On the other hand mono-, di-, and triglycerides are ? beside of many other organic compounds ? a mayor part of native lecithins from soy, sunflower, canola etc.. and low amount by-products in de-oiled and processed lecithins. Again the combination of 1H-NMR and 31P-NMR as well as 13C-NMR enables its qualitative and quantitative analysis. Method validation and several applications are presented in the study.

Supercritical Fluid Purification of Lisophosphatidylcholine from a Phospholipase A2 Hydrolysate.
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Hydrolysis of Hydrogenated phosphatidylcholine catalyzed by a food grade phospholipase A2 has been studied. The main purpose of the present work was to obtain a mixture comprised of lysophosphatydilcholine and free fatty acids with a content of the original phosphatidylcholine below 5 %. Different variables such as temperature, ratio of water to phospholipids and amount of enzyme were studied. The reaction was effected in aqueous medium in absence of organic solvents. Supercritical fluid extraction has been utilized to separate free fatty acids from lysophosphatidylcholine in a plant comprised of one extraction cell and two separator cells where a cascade decompression takes place. However, water from the hydrolysis reaction was not removed from the mixture prior to fractionation to test its effect on the free fatty acids extraction. Pressure, temperature, and amount of CO2 were also considered. Besides supercritical fluid extraction, other methodologies such as crystallization and accelerated solvent extraction have been also considered. The purified lysophosphatidylcholine obtained is intended to be used as a precursor in the enzymatic synthesis of structured phospholipids with specific fatty acid at sn-2 position.

Phospholipid Species and Minor Sterols in Human Milks.
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The purpose of the study was to characterize the phospholipid and sterol composition in four French breast milk samples, with an aim to highlight molecular lipid species of possible nutritional significance for infants. Main PL species were phosphatidylethanolamine (PE, 21.3 ±4.7%), phosphatidylcholine (19.0 ±2.2%) and sphingomyelin (43.3 ±2.6%). PE contained more arachidonic acid (4.8%) than other PL species ($P<0.001$). PE and PS + PI contained the highest proportion of DHA among PL species (0.94% and 1.13%, respectively, $P<0.05$). Several minor bioactive sterols were detected in the polar lipids of human milks, e.g., desmosterol, lathosterol, lanosterol, stigmasterol and β-sitosterol. The metabolic significance and health impact of such lipid consumption by the infant should thus be explored.

Technical Program