2013 Annual Meeting Abstracts

Phospholipid

MONDAY

AFTERNOON

BIO 1.1/PHO 1: Polar Lipids: Chemistry, Technology, and Applications
Chair(s): X. Xu, Wilmar Global R&D Center, China; Aarhus University, Denmark; M. Ahmad, Jina Pharmaceuticals Inc., USA

Enzymatic "green" Preparation of Sugar-fatty Acid Esters

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Saccharide-fatty acid esters are an emerging category of biobased surfactants prepared entirely from renewable resources that are used as emulsifiers in foods, cosmetics, and pharmaceuticals, and possess anticancer and insecticidal properties. Typically, the esters are prepared chemically under harsh condition: temperatures near 200 C, employment of solvents, etc., which can cause undesirable side-reactions and produce waste products. Our group has been investigating the use of lipases and novel bioreactor system design to prepare sugar esters under solvent-free conditions and relatively low temperature: ~65 C. Using a stoichiometric feed of saccharide and fatty acid, our approach achieves 90-95% pure ester on a 10-30 gram scale, which, due to the absence of excess reactants and solvent, will require little or no further downstream purification to achieve industrial specifications. The presentation will provide an overview of our recent work, including an evaluation of its physical properties.

Deep Eutectic Solvents: new Opportunities for Lipase-catalyzed Reactions

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In recent years, researchers focused on finding green alternative media to organic solvents for enzyme-catalyzed reactions. Thereby, ionic liquids (IL) emerged as fascinating media for biotransformation. However, one drawback to their wider development is their cost, synthesis and purification. Recently, a novel medium with similar properties to IL but with additional advantages regarding cost, environmental impact and synthesis has been created: Deep Eutectic Solvents (DESs). DESs result from the association between an ammonium or phosphonium salt with a hydrogen-bond donor. Results showed the superior performance of choline chloride pair with urea or glycerol over other types of DESs in improving the conversion and selectivity of alcoholysis reaction of aliphatic ester using Candida antarctica B lipase (iCALB). We demonstrated that some DESs can react and compete with the substrates in alcoholysis reactions leading to byproduct formation and DES destruction. Although we know that iCALB denaturates in solutions of urea, it did not denaturate quickly in DESs containing urea or glycerol and its stability is sufficient to allow the reaction for several days. Finally, we opened new perspectives on the enzymatic modification of polar subtrate with this new generation of green, cheaper and easy to handle solvent in binary mixture with water.

Highly Efficient Synthesis of Phosphatidylserine in a Novel Medium

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Recent reports have shown that phosphatidylserine (PS) supplemented in the diets play an important role in preventing Alzheimer's dementia, improving memory, increasing attention and relieving depression. PS synthesis via enzyme-mediated transphosphatidylation of phosphatidylcholine (PC) with L-serine has been gaining more attention due to mild reaction conditions and environmental friendliness. Generally, this reaction is carried out in a biphasic system or a purely aqueous system. A serious drawback of these systems is that they contain considerable amounts of water, which results in the undesirable hydrolysis of PC and PS to form byproducts. A novel reaction system for enzymatic synthesis of PS was reported in this work. Herein, ?-valerolactone which has recently been described as an excellent candidate of green solvent available naturally was employed as the reaction-medium. Our results indicated that, under the optimized reaction conditions (i.e.: 40°C; substrate molar ratio (L-serine/PC) 3; 60 U Phospholipase D from Streptomyces chromofuscus; 12 h), PS yield could be achieved to 95% combined with no byproduct formation. In particular, the present work accommodated a facilely and efficiently enzymatic strategy for preparing PS, which possessed obvious advantages over the reported processes in terms of high efficiency.

Role of phospholipid in formation of nanoemulsions of bioactive lipids

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Bioactive lipids are defined as changes in lipid moities that result in functional consequences. Their bioactivity makes them very sensitive natural deterioration and so the use and delivery system of bioactive lipids should be critically planned so as to obtain the full benefits of it. Presently, Nanoemulsion-based delivery systems represent an effective approach to improve the dispersion of the bioactives into food products, to protect them against degradation or interaction with other ingredients, to reduce the impact on organoleptic properties of the food and to improve their bioavailability. The formation of very fine emulsions in the nanometric range (< 200 nm) can be achieved by high pressure homogenization at low temperature in presence of a suitable emulsifier which can increase its kinetic stability and minimise the impact on the organoleptic properties. In this study, an attempt was made to produce nanoemulsion of conjugated linolenic acid (CLnA) rich oil by using egg phospholipid as emulsifier; as phospholipid and CLnA can show synergistic protective effect in human system. The two forms of phospholipids were used as emulsifiers, phospholipid and lysophospholipid, and the efficiency of the two emulsifiers to prepare nano-emulsions was analyzed. The droplet size and zeta potential of the two types of nanoemulsions were determined after just preparation of emulsions and after one month of storage.

Microencapsulation of Krill oil Using Complex Coacervation

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The research work was aimed at the optimization of a bioprocess to yield gelatin/gum arabic multinuclear

microcapsules of krill oil (KO), via complex coacervation. Initial screening experimental work was performed to identify the parameters that have the most significant effects, including the homogenization speed, ratio of core to wall materials (RCW), concentration of wall materials (CWM), pH and stirring speed on the encapsulation efficiency (EE) of KO. On the basis of the results of the screening trials, a three-level-by-three factor Box-Behnken design was used to evaluate the linear, quadratic and bilinear effects of RCW (1.25:1 to 1.75:1), pH (3.8 to 4.2) and stirring speed (2 to 4, over a scale of 10) on the EE of KO. The optimal predicted conditions for the microencapsulation of KO, were RCW (1.75:1), pH 4 and stirring speed of 2, with a 90.6% of EE. The chromogenic red-orange color of KO, conferred by astaxanthin, facilitated the stereomicroscopic visualization of the entrapped oil and without the need of a lipid-soluble dye. The microcapsules, formed by complex coacervation, were circular in shape and had sufficient stability to maintain their structure, in the absence of any cross-linking agent.

Improved Acylation of Phytosterols Catalyzed by Candida Antarctica Lipase a With Superior Catalytic Activity

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Technical processes of chemical esterification presently used for the preparation of steryl esters are generally performed at high temperature in the presence of chemical catalyst, accompanying with high energy consumption, browning of products and low selectivity. This work reported a novel approach to synthesize phytosterol (?-sitosterol as a model) fatty acid esters by employing immobilized CAL A which shows a superior catalytic activity to other immobilized lipases including CAL B, Lipozyme NS-40044 TLL and Lipozyme TL IM. CAL A achieves 6-14 times faster esterification of ?-sitosterol with myristic acid than other lipases. The effects of enzyme concentration, fatty acid types, substrate molar ratio, reaction temperature and time, and polar/non-polar organic solvents were investigated. A series of ?-sitosteryl fatty acid esters (C2-C18) have been successfully prepared with structural identification of products by 1HNMR and Fourier transform-infrared spectroscopy (FTIR). CAL A showed low activity towards short chain fatty acids (C2-C6) but it increased significantly in the presence of fatty acid anhydride counterparts. CAL A rendered remarkably high activity for medium and long chain fatty acids (? C8). An increase in double bond in fatty acid molecule reduced the esterification activity of CAL A. Reaction time, temperature, enzyme load, substrate ratio and concentration, and solvent property are found to profoundly influence reaction rates. 93-98%Yield of ?-sitosteryl esters could be achieved with hexane as solvent, fatty acid (C8-C18)/ ?-sitosterol (1:1, mol:mol), 5-10% CALA load at 40-50°C for 24h. This work demonstrated the promising potential of CAL A in bioprocess of phytosterols for value-added application.

TUESDAY

MORNING

PHO 2: Krill Lipids Analysis, Algal Oil
Chair(s): B. Diehl, Spectral Service AG, Germany

NMR of Krill Oil, The USP Accepted Method for Identity and Quality Control

B. Diehl[(1)]
[(1)]Spectral Service AG, Germany

A special part of the lecture will be the material accountability study of krill oil by a combination of 1H, 31P and 13C NMR spectroscopy. The mayor compounds of krill oil are phospholipids (including several types of ether-lipids) triglycerides as well as mono- and diglycerides, free fatty acids, cholesterol and phytol esters. In addition minor components like homarin and its degradation product is marker for the identity of krill origin. The combination of NMR and HPLC/MS completes the identity and adulteration tests. It is shown that 31P NMR is the most powerful and valid method to analyse any lecithin[1] source (soy bean, egg, sunflower, milk etc. and especially the marine types like fish roe and krill). A full validation of the 31P NMR method is presented.

CANCELLED-Lipids and Lipolytic Enzymes of Microalgae

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[(1)]University of Maine, Laval, FRANCE, France

Isolation and Analysis of Ether Lipids from Krill Oil

G. Randel[(1)]
[(1)]Spectral Service, Germany

Krill oil is different from other lipid mixtures of marine sources. Especially the asymmetric fatty acid composition in triglycerides and phospholipids is remarkable. However, there are several other characteristics of the krill phospholipid composition. The amount of ether phospholipids is a good identity criterion for krill origin. 9?12 MOL% of the total PC content is represented by PC ether. A chromatographically separation of PC and PC ether as well as other species and its ether types is not possible due to the similarity of both lipid types. A distinction in HPLC/MS (SIM mode) also is a problem as long the molecular composition and differences of the fatty acid composition in PC and PC ether are not known. The use of different types of phospholipases (A1, A2 and lyso-phospholipases) now enables a successful sequential degradation of PC to LPC and GPC while PC ether is not involved in this enzymatically reaction. Pure PC ether and LPC ether could be obtained and characterized by NMR and HPLC/MS techniques. In addition double lyso-APE was obtained and the fatty acid composition of the N-acyl groups could be clarified.

From Algae Farm to Human Nutrition Products- Sustainable and Dosage-Controlled EPA Omega-3 with Polar Lipids

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For many years, phototrophic microalgae have been identified as rich sources for Omega-3 oils. Qualitas Health, through selective breeding and optimized cultivation systems, has successfully refined the growth of Nannochloropsis microalgae to produce a highly bioavailable form of Eicosapentaenoic acid (EPA) in a variety of lipid classes-phospholipids, glycolipids, triglycerides, and free fatty acid. Extraction and refinement methods have been perfected to yield both high concentrations of EPA Omega-3 and EPA conjugated to the polar lipids. Recent in-vivo animal trial and human nutrition testing shows that the bioavailability of a mixture of polar lipids and EPA is equivalent to the bioavailability of krill oil. This Omega-3 form is, thus, a vegetarian, krill-like product that can be produced both

economically and sustainably. Results of extraction, refinement, animal trials, and human trials will be presented.

AFTERNOON

PHO 3: Phospholipids for Industrial and Feed Applications
Chair(s): B. Sebree, Archer Daniels Midland Co., USA; M. Rebmann, Perimondo LLC, USA

Functionality and Applications for Phospholipids in Feed and Industrial Products

B. Sebree[(1)]
[(1)]Archer Daniels Midland Co, United States of America

Phospholipids have been used for many years in feed and industrial applications for nutritional, as well as unique technical functions. Commercial sources of phospholipids, such as soy lecithin, are relatively inexpensive naturally sourced molecules that exhibit functionalities not found in other emulsifiers and surfactants. Soy phospholipids with their inherent amphoteric character have the ability to form various structures make them valuable and interesting molecules to exploit. This talk will review the basic theory behind the unique technical functions that phospholipids provide in these application areas, as well as nutritional aspects provided to feed products. Major applications in feed and industrial systems will be discussed as well.

Phosphatidylcholine liposomes as boundary lubricants with extremely low friction coefficients

R. Goldberg[(1)]
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Using a surface force balance we have studied the forces between surfaces covered with liposomes across aqueous media. Pure SUVs of hydrogenated soy phosphatidylcholine (HSPC) lipids, which adsorbed spontaneously onto the negatively charged surface were found to be excellent lubricants. In pure water, these vesicles are capable of reducing sliding friction coefficients ? to uniquely-low values ? ?10-4 ? 2x10-5 at pressures of over 100 atmospheres. The lubrication effect itself is attributed primarily to the hydration layers surrounding the outer phosphocholine (PC) headgroups of the vesicles. These hydration water molecules are tenaciously attached, yet labile, and can therefore provide a ball-bearing like effect. This arises because the water of hydration can sustain a large pressure without being squeezed out from between the surfaces, while at the same time the hydration shells relax rapidly. The hydrated PC layers display an extreme stability to pressure when liposomes are closely packed on the surface and in the solid-gel phase. Measuring the friction coefficient for liposomes in their liquid phase provided poor lubrication (friction coefficients up to ? ? 0.1) at pressures of a few MPa. Tribometer measurements of friction between a polyethylene surface and the metallic head of a joint implant carried out across different Multi-Lamellar-Vesicles suspensions. Results showed the shear reduction capability of the gel-phased liposomes. while the friction coefficient μ in the saline measurements was in the range of 0.17 ± 0.025, the gel-phased liposomes, in particular the HSPC and DSPC showed dramatic shear reduction of an order of magnitude μ

Novel Bio-based Dispersants for Coating Applications

S. Baseeth[(1)]

[(1)]ADM, United States of America

Latex paints and coatings are popular consumer paints as they are easy to apply, are usually easy to clean up, nonflammable, generally lack a disagreeable odor and can be used on both interior and exterior surfaces. 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. These new paints are durable, cost-effective and less harmful to human and environmental health. New bio-based dispersant technology developed by ADM is based on non-polymeric amphoteric phospholipid chemistry derived from renewable platform. The products contain >95% bio-based carbon content. This unique technology performs very similar to traditional anionic workhorse polymeric dispersants. Being amphoteric our dispersants are able to tune to the changes in the pH and ionic environments which is very unlikely with common phospholipids. This allows our dispersants very versatile performance irrespective of the changes in the nature of surface treatment resulting in very acidic to basic pigment. Lower dispersant demand and ability to minimize surfactant use supplies an overall net result of paint cost reduction, improved mechanical properties such as scrub or water resistance. In this paper results of studies comparing the new amphoteric dispersant technology to conventional anionic dispersants in white tint bases are reported. Results show improved color acceptance, stability, rub-up resistance, and mechanical properties. An overall reduction of the formulation cost per gallon was also obtained.

The Effects of Soybean Phospholipids and N-3 and N-6 Fatty Acids on Growth, Body Composition, Lipid Metabolism, and Delta-6-Desaturase Gene Expression in Channel Catfish and Largemouth Bass

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[(1)]University of Arkansas at Pine Bluff, United States of America [(2)]UAPB, United States of America

Channel catfish were fed a control diet with 4.3% endogenous phospholipids (no supplements), or two other diets with 2 or 4% added soy lecithin in a feeding trial to determine performance effects. Lecithin did not affect survival, growth, feed consumption, whole-body total lipid, hepatosomatic index, innate immune response, plasma cholesterol or triglycerides. Supplemental lecithin improved feed conversion (FCR), increased whole-body protein, phosphatidylcholine, and hepatic glycogen, and decreased hepatic lipid and total phospholipid (PL). The dietary PL requirement, if any, of catfish for growth and survival is < 4.3% (1.5% phosphatidylcholine). We determined the effects of dietary n-3 and n-6 lipids alone, or in combination with PL on performance and ?-6 desaturase gene expression in catfish and largemouth bass (LMB). PL alone did not affect survival or FCR of bass, but PL plus n-6 lipids improved FCR comparably to n-3 lipids. LMB fry fed supplemental PL grew faster up to 3.67 g, regardless of n-3 or n-6 lipid source, indicating a dietary requirement for PL. ?-6 desaturase was poorly expressed in small and larger bass fry compared to younger, smaller channel catfish. This is consistent with the apparently higher dietary requirement for long-chain polyunsaturated fatty acids in LMB compared to catfish.

Enhancement of reproductive performance of Gangetic leaffish (Nandus nandus) and Gourami (Colisa fasciatus) with dietary phospholipids

Z. Hossain[(1)]
[(1)]Manitoba University, Canada

In the present study sperm quality, histological structure of the liver and developmental stages of ovary, level of Ca2+ concentration, embryonic development and larval growth were investigated for the confirmation of the positive effects of PUFAs in reproduction and gonadal maturation of Gangetic leaffish, Nandus nandus and Gourami, Colisa fasciatus. Treated group was fed 1% squid extracted phospholipid supplemented diet whereas controlled group was fed the same

except phospholipid. In comparison to the control group, treated group exhibited higher gonadal maturation which resulted in spontaneous spawning. The live sperm count was significantly higher (P<0.01) in treated group compared to control group. During the pick breeding (April) season in case of treated group most of the oocytes were found at nuclear migration and tertiary yolk oocyte stage while in case of control group most of them were found in primary and secondary yolk oocyte stage. During spawning season lipid granules and normal morphological structures of hepatocytes with enlarged nucleus and considerable amount of vacules were observed in case of phospholipid treated fish liver whereas less lipid granules with scattered necrosis and large vacuoles in cytoplasm with polarized nucleus were observed in control group. The serum Ca2+ concentration in treated females were significantly higher (P<0.05) in contrast to the controlled females of both the fishes during the breeding season. The experiment suggests that supplementation of dietary PUFAs eventually improve the spawning performances of fish.

WEDNESDAY

MORNING

PHO 4: General Phospholipids
Chair(s): S. Jadhav, Archer Daniels Midland Co., USA; M.C. Tomás, CIDCA-UNLP, Argentina

Impact of an addition of phospholipids as emulsifiers on structural and rheological properties of a cream cheese model.

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Cream cheese is an oil-in-water emulsion with high-fat content (33 %) stabilized and textured by protein network. The aim of this study is to investigate the impact of an addition of two concentrations of natural milk phospholipids as emulsifiers on structural and rheological properties of a cream cheese model. The emulsion was studied at each step of the process (heat treatment, acidification and homogenization). The increase of phospholipids concentration led to a decrease of emulsion viscosity at each intermediary process step and to a decrease of final cream cheese firmness. The addition of 0.5 % of phospholipids in fat induced products twice less firm than standard products. Moreover, the addition of phospholipids affected the fat globule size, especially after the homogenization. Indeed bigger fat globules are observed when products are enriched in phospholipids, and some of them coalesce. These texture modifications can be explained by the analysis of final products structure by confocal and transmission electronic microscopy. It showed a competition between proteins and phospholipids at the fat globule interface and a displacement of proteins in favour of phospholipids. Phospholipids at the interface didn?t interact with proteins, it decrease network strength then product firmness and modify sensory properties.

Biological Properties of Deoil Sunflower Lecithin

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In the last time, large attention is spared to the sunflower lecithin. It is determined by the fact that content of phospholipides in sunflower seeds practically does not differ from the soya beans, their extraction requires the less

protracted temperature treatment and also more fatty acids are contained in them. Regular use of deoil sunflower lecithin during one and a half or two and more months caused the substantial change of liver functioning. It showed up in diminishing of bile condensing, declining of cholesterol crystals, calcium bilirubinate, bilious salts, and microlites content in it, its colloid stability improvement, micelle formation increase, diminishing of the phenomena of fatty infiltration. The natural antagonism of PC (other phosphatides) and cholesterol is the basis of lecithin effect on the metabolism of fats. Ability of cholesterol to promote inflexibility of cytoplasm membranes and increase their hardness shows up already at the level of cytoplasm membranes, while PL, including PC, make them more fluid and liquid, that is increase functional activity of membranes. Regular use of lecithin results in the decline of level of cholesterol in blood and blood vessel walls, and also promotes ability of bilious acids to excrete this compound from blood flow.

Multiscale Molecular Simulation and Experimental Investigation of Sunflower Phospholipids Liposomes Using for Scavenging Free Radicals

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Creating artificial membrane liposomes is one of the promising areas of modern nanobiotechnology. The membrane properties of the liposomes can be responsible for various phenomena such as immobilization of protein, diffusion and transport of bioactive compounds By using simulations based on quantum mechanical and molecular dynamics methods, the stable structure, electronic and dynamical properties of liposomes will be investigated. In addition, the formation mechanism of liposomes with lipid bilayer in a medium with the solvent (from water) will be elucidated by the molecular simulations. The research devoted to antiradical and antioxidant properties of the sunflower lecithin and its components such as phosphatidylcholine, phosphatidyletanolamine and other phospholipids will be undertaken, because possible using of these products in food technologies. This direction may include investigation of reactivity of lecithin from seed sunflower oil and its ingredients, towards reactive oxygen species and other free radicals. Highly reactive oxygen species and lipid peroxyl radicals are produced in living cells in course of aerobic metabolism and are involved in a number of life sustaining biochemical processes. The failures of the protective antioxidant systems of the cells lead to oxidative stress causing cardiovascular disease, cancer, chronic inflammatory and other pathologies. Therefore, there is a demand for new antioxidants and antiradical agents, which may be used in vivo for scavenging free radicals. The new products from sunflower seeds are expected to be able to react with free radicals and can be efficient antioxidant agents

Hazelnut oil as a source of lecithin

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The overwhelming majority of lecithins are produced from soya beans and some quantity is produced from egg yolk. However, lecithins are known to be contained in other oily produces, in particular sunflower and hazelnut. Taking into account the fact that lecithin can be produced from hazelnut being non-GMO and low pesticide produce, in the present study both laboratory and industrial production of hazelnut lecithin have been investigated. Total hazelnut production of Turkey is estimated to be 500,000 tonnes per year (FAO 2010) which ranks Turkey as the top hazelnut producing country in the world. Hazelnut contains 60 % of oil by weight. The compositional study on the hazelnut oil showed that it is rich in oleic acid (84.52 g/100g), ?-sitosterol (97.16 mg/100g) and ?-tocopherol (336.15 mg/kg) making it a nutritionally valuable edible oil. The soap-stock which is a by-product of hazelnut oil production is mainly used in feed and cosmetic industry. However our results showed that it can be effectively used as a source for lecithin production. It was found that crude hazelnut contains 1.00 % of phospholipids. The results of this study also showed

that major phospholipids found in hazelnut lecithin were phosphotidylcholine (PC), phosphotidylethanolamine (PE) and phosphotidylinositol (PI) which account almost 55 %, 28.8% and 12.3 %, respectively.

Functional Properties of By-products Obtained by Enzymatic Degumming of Crude Soybean Oil

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The enzymes used in degumming, called phospholipases, specifically act on phospholipids without degrading the oil itself. Degumming using a phospholipase C enzyme allows to meet all market especifications while it increases the oil yield. The aim of this study was to evaluate antioxidant and emulsifier properties of by-products from the enzymatic oil degumming process (LYG) subjected to modifications as deoiling (LYG deoiled) or ethanol fractionation (LYG soluble, LYG residue). LYG soluble allowed to obtain more stable O/W emulsions (30:70 wt/wt) in comparison with those by-products assayed at different concentrations (0.1-1.0%). Also, Deoiled Soybean Lecithin (DSL) and LYG deoiled had a similar behavior in relation to the kinetic destabilization (%BS profiles), despite the different degumming processes used. The study on induction times (Metrohm Rancimat) showed a significant antioxidant effect ($p < 0.05$) against a refined sunflower oil associated with all the by-products analyzed. However, LYG soluble and DSL showed a strong effect on the oil stability at high concentrations (1000-2000 ppm). These results showed that LYG deoiled and LYG derivates obtained by ethanol fractionation are a potential alternative for industrial application as an additive.

Self-Assembled Structures of Phospholipids: A key to Multifunctional Orgaongel

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[(1)]Archer Daniels Midland Company, United States of America [(2)]Archer Daniels Midland Company, United States of America [(3)]Archer Daniels Midland Company, United States of America

Phospholipid-based organogels are a unique class of functional soft-materials. They primarily consist of a continuous non-polar phase, small amount of dispersed water phase and a network of well-defined and high-aspect-ratio phospholipid aggregates. The non-polar and polar phases are entrapped by the network. The propensity of phospholipid to hydrate in presence of water triggers hierarchically self-assembly with the aid of non-covalent interactions to form aggregates. Such organogels are viscoelastic, thermodynamically stable, stimuli-responsive, structurally well-ordered, optically clear and more importantly biocompatible. Due to their structural and functional benefits they exhibit tremendous potential for diverse applications; however, their utility has been successfully applied only in the pharmaceutical field. One of the primary obstacles has been the requirement of highly purified phospholipid (at least 80-95% phosphatidylcholine content). With a view to overcome this barrier a novel method was developed to enable usage of phospholipid to yield commercially and economically viable organogel without sacrificing the attributes of a typical phospholipid organogel. The mechanism of formation of a gel was thoroughly studied by using various methodologies such as rheology, optical microscopy and small angle X-ray scattering. Details of the study are discussed in this presentation. The developed method enabled us to expand the utility of a phospholipid organogel to food, feed, cosmetics and industrial applications. Possible applications are explored and briefly discussed in this presentation as well.

Phospholipid Poster Session

Optimization of Phospholipase A1-Catalyzed Hydrolysis of Phosphatidylcholine for Producing Lysophosphatidylcholine by Response Surface Methodology

C. Lim[(1)], I. Kim[(2)], M. Lee[(3)], B. Kim[(4)]

[(1)]Chung-Ang University, Korea, Republic of [(2)]Korea University, Korea, Republic of [(3)]Ilshinwells Co., Ltd., Korea, Republic of [(4)]Chung-Ang University, Korea, Republic of

The aims of this study were to model the enzymatic partial hydrolysis of soy phosphatidylcholine (PC) in hexane for producing lysophosphatidylcholine (LPC) and to optimize the reaction conditions by response surface methodology. Of the 12 kinds of commercial lipases and phospholipases tested, phospholipase A1 from Thermomyces lanuginosus/Fusarium oxysporum was selected as the suitable biocatalyst for the hydrolysis. The effects of temperature (40-60 °C), reaction time (1-7 h), water content (10-30% of the weight of PC), and enzyme loading (1-7% of the weight of PC) on the contents of PC, LPC, and glycerylphosphorylcholine (GPC) in the reaction products were elucidated using the models established. Optimal reaction conditions for maximizing the content of PC while suppressing the formation of GPC were: temperature, 58 °C; reaction time, 4 h; water content, 22%; enzyme loading, 1%. The contents of PC, LPC, and GPC were 5.3 mol%, 92.6 mol%, and 2.1 mol%, respectively, under these conditions. The LPC produced was mainly comprised of linoleic (76.8 mol%), oleic (13.2 mol%), and linolenic acids (6.8 mol%).

Release of Fatty Acid Hydroperoxides and Hydroxides From Lipoprotein Phospholipids by Group Iia, v and x of Human Secretory Phospholipases (spla2s)

A. Kuksis[(1)], W. Pruzanski[(2)]

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The objective of the study was the demonstration of hydrolysis of hydroperoxides and hydroxides of plasma lipoproteins by group IIA, V and X sPLA2s. Auto-oxidized preparations of high (HDL) and low (LDL) density lipoproteins containing 15-50 nmoles/mg protein of hydroperoxides and 5-25 nmoles/mg of protein of hydroxides were used. Total HDL, HDL3 and LDL were digested separately using varying substrate/enzyme ratios. Digestions were performed for 1-4 h at 37 oC in a total volume of 1 ml Tris/HCl buffer, pH 7.5, containing 10 mM CaCl2 and 0.01% BSA, using 0.1-2.5 µg sPLA2/mg protein. The reactions were stopped by adding the extracting solvents (CHCl3/MeOH, 2:1 v/v). Normal phase LC/ESI-MS analyses were performed as previously described. It was shown that the hydroperoxides and hydroxides of PtdCho of HDL and LDL were extensively hydrolyzed in 1 h by group V and X human sPLA2s using 1 µg sPLA2/mg protein. In contrast, group IIA sPLA2 required 4 h and 2.5 µg enzyme/mg protein for comparable hydrolysis. It is concluded that human sPLA2s, especially those of group V and X, are capable of effective hydrolysis of the hydroperoxides and hydroxides of plasma HDL and LDL, which had not been previously shown. None of these sPLA2s showed the preferential hydrolysis of oxo-PtdChos demonstrated previously for snake and bee venom sPLA2s.

Modeling simple lipid phase separation and effects of amphiphilic molecules on lipid domains

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We developed an algorithm allowing the visualization of lipid phase separation and the effects of any amphiphilic molecule on the system. The method is based on the previously published Hypermatrix method (Brasseur et al, 1987). The molecules of interest (lipids, surfactants, ?) are first oriented at the interface and for each pair of molecules, the interaction energies are calculated for a large number of positions (typically more than 107). Those energies are stored in a matrix which is then used in the construction and minimization of the system. To visualize lipid separation, we typically construct a grid of 360 000 molecules (600 X600 molecules), initially positioned at random. The energy of the system is calculated using the calculated matrix. The energy of one molecule is equal to the sum of the energies with its 24 closest neighbours in the grid. Random permutations are made and the energy of the new configuration is calculated. By a monte carlo procedure, this new configuration is kept or not, as a function of the energy difference between the two states. This method was notably successfully applied to simple lipid systems, such as DOPC/DPPC, with or without surfactant such as lipopeptides (Deleu et al, 2013). References 1. Brasseur, R., et al., BBA, 1987. 903, 11-17. 2. Deleu, M et al, BBA, 2013, 1828, 801-815

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