PHO 1: General and New Sources of Phospholipids and Applications

Chairs: Mabel Tomás, CIDCA (CONICET-UNLP), Argentina; and Swapnil Jadhav, Archer Daniels Midland Co., USA

Characterization of Glycerophospholipid Molecular Species in Marine Edible Clams by Using HPLC-ESI-MS/MS Dayong Zhou*[1], Zhongyuan Liu[2], Fawen Yin[2], Liang Song[2], Qi Zhao[2], and Beiwei Zhu[2], *[1]Dalian Polytechnic University, China; [2]College of Food Science & Technology, Dalian Polytechnic University, China*

Objective: Clam is one of the important aquatic economic animal, which ranks the first in production in shellfish species. This study was carried out to analyze lipid class compositions and molecular species profiles of glycerophosphocholine, glycerol-phosphoethanolamine, glycerophosphoserine, glycerophosphoinositol, lysoglycero-phosphocholine, lysoglycerophosphoethanolamine and lysoglycerophosphoinositol of lipids extracted from six species of major edible clams in the Bohai Sea and Yellow Sea of China, which provides basic data for utilization of the lipid resource from clams. Methods Used: The lipids were extracted by using a mixture of ethanol and hexane (v/v, 1:1), lipid class composition was measured by using thin layer chromatography-flame ionization detection, and glycerophospholipid (GP) molecular species was analyzed by using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Results: Results indicated that the dried meat of Cyclina sinensis, Mactra chinensis Philippi, Mactra veneriformis Reeve, Meretrix meretrix, Ruditapes philippinarum, and Saxidomus purpurata contained 10.12, 5.10, 5.49, 9.05, 7.29 and 6.65% lipids, respectively. The lipids recovered from clams were composed of triacylglycerol, diacylglycerol, monoacylglycerol, phospholipid (PL), free fatty acids and cholesterols. Among them, PL constituted the majority of the lipids from clams (39.86-74.05% of total lipids). At least 514, 516, 602, 518, 511 and 511 GP molecular species were characterized, respectively, in lipids from Cyclina sinensis, Mactra chinensis Philippi, Mactra veneriformis Reeve, Meretrix meretrix, Ruditapes philippinarum and Saxidomus purpurata. Most of the predominant GP molecular species in clams contained omega-3 polyunsaturated fatty acids, mainly eicosapentaenoic acid and docosahexaenoic acid. Conclusion: Our research demonstrated that clam is a potential resource of GP enriched n-3 PUFA.

Facilitating Phospholipids Analysis in Complex Matrices by Using Automated Routines Y.B. Monakhova*, B.W.K Diehl, and E. Zailer, *Spectral Service AG, Germany*

Nuclear magnetic resonance spectroscopy (NMR) is a useful tool to quantitate phospholipids (PLs) in various matrices. This technique has clear advantages in simplicity and speed over traditional methods (e.g., HPLC, ESI-MS, MALDI-TOF/MS). The method is characterized by a good specificity and multiple standards are not required for quantification. However, signal overlap and difficulties in spectra interpretation prevent routine use of NMR in analytical practice. To facilitate and simplify NMR analysis of phospholipids in complex mixtures specialized routines aimed at automated quantification have been proposed. The development of approaches of automated processing of NMR data remains virtually unexplored. Our purpose was to increase performance and simplify the analysis of large amounts of digital data recorded by a spectrometer. In particular, automated routines were written in MATLAB environment for quantification of PC, LPC, PE, APE, LPE and other PL species in several types of krill oil matrices as well

as PC, PI, LPC, PS, PE, DPG, PG and LPE in soy and sunflower lecithin. In both cases triphenyl phosphate was used as an internal standard. The software includes data import, mathematical preprocessing, integration, and generation of a report file using template. The duration of automated NMR data processing does not exceed 1 min per sample. It was possible to precede about 50% of our routine samples without human intervention.

Characterization of Chia Microencapsulated Oil from Freeze-dried Layer-by-Layer Emulsions with Sunflower Lecithin Luciana M. Julio[1], Claudia N. Copado[1], Vanesa Y. Ixtaina[1], Susana M. Nolasco[2], and Mabel Tomás*[1], *[1]CIDCA (CONICET-UNLP), Argentina; [2]TECSE (Fac. Ing.-UNCPBA), Argentina*

Microencapsulation is an alternative to protect chia oil from the adverse conditions of chemical environment, since its susceptibility to oxidation due to their high content of PUFAs. The aim of this work was to obtain and characterize chia oil microcapsules from freeze-dried layer-by-layer emulsions (LBLE). A lecithin (Lec) (-35mV at pH 3) and Lec+chitosan (LCh) (+45mV at pH 3) stabilized emulsions with maltodextrin addition were prepared by homogenizing (600 bar, 4 cicles) 5% wt chia oil with 95% wt aqueous solution and subsequently freeze-dried. The powders were stored in closed chambers at HR 33%, 25±2ºC and characterized in relation to moisture content, water activity, particle size, microstructure and oxidative stability. The D [3,2] mean diameter ranged from 1.2 to 2.7µm and the encapsulation efficiency was ~80% in all cases. Water activity and moisture content of powders were around 0.4 and 1.7%, respectively. Microcapsules presented porous, irregular and glass like surfaces. Both powders exhibited higher oxidative stability than no encapsulated chia oil giving LCh microcapsules the better protection against lipid oxidation. These data suggest that multilayer microcapsules could be an effective technology to deliver chia oil into functional foods.

Chia Seed Oil Bi-layer Emulsions with Modified Sunflower Lecithin Luciana M. Julio[1], Claudia N. Copado[1], Susana M. Nolasco[2], Vanesa Y. Ixtaina[1], and Mabel Tomás*[1], *[1]CIDCA (CONICET-UNLP), Argentina; [2]TECSE (Fac. Ing.-UNCPBA), Argentina*

There is a high trend for development edible delivery systems to enrich, protect and release bioactive lipids within foods. Also, modified sunflower lecithin presents good O/W emulsifying properties which are obtained by fractionation process. Multilayer O/W emulsions with sunflower phosphatidylcholine-enriched fraction (PCF) and chia oil (high omega-3 content) would be an interesting option. Mono (PCF) and bilayer chia O/W emulsions (PCF+chitosan-Ch) were prepared homogenizing 5%wt chia oil with 95% wt aqueous solution (600bar, pH3) and stored ~1 month at 4.0±0.5°C. Emulsions were evaluated by confocal microscopy, ζ-potential, apparent viscosity, the evolution of backscattering profiles, particle size distribution, mean diameter (D[3,2]) and peroxide value (PV). The inversion charge from -36 to +54mV was observed with the addition of chitosan. The particle size distribution was monomodal for the two-layer whereas the mono-layer emulsions presented a very little shoulder.D[3,2] was 0.24µm and 0.33µm for mono and bi-layer emulsions, respectively. Some signs of creaming after 2 weeks of storage were noticeable for mono-layer emulsions, while bi-layer emulsions were physically stable during this period.Regarding lipid oxidation, PCF+Ch systems were more stable than those with PCF alone. These data suggest that bilayer (PCF-Ch) O/W emulsion constituting a good alternative to protect and deliver chia oil into functional foods.

PHO 2: Chemical and Enzymatic Synthesis of Phospholipids

Chairs: Moghis Ahmad, Jina Pharmaceuticals Inc., USA; and Xuebing Xu, Wilmar Global Research and Development Center, China

Synthesis and Characterization of a Novel Array of Polyphenol-containing Phospholipids: A Physicochemical Study Sampson Anankanbil*[1], Zheng Guo[2], and Bianca Perez[1], [1]*Dept. of Engineering, Aarhus University, Denmark;* [2]*Aarhus University, Denmark*

Lipid autoxidation in emulsions is postulated to occur as a result of interactions between trace transition metals in the aqueous phase and preformed lipid hydroperoxides located at the oil-water interface. As the main barrier to prevent the diffusion of oxygen and free radicals, the thickness, molecule packing and mechanical stability of interfacial layer are governing the physical and oxidative stability of the emulsion. Hence, a surface-active molecule containing a phenolic moiety might be an ideal surfactant to function as both emulsifier and antioxidant to trap diffusing free radicals at the interface. To this end, a new homologous series of amphiphilic lipids were synthesized through the acylation of phospholipids with phenolic acid derivatives. The resulting products were structurally identified and characterized by means of mass spectroscopy, Fourier transform infrared spectroscopy (FTIR), and 1H and 13C nuclear magnetic resonance. A pronounced structure-property relationship was established through Differential Scanning Calorimetry (DSC) and Langmuir monolayer analysis in correspondence to their molecular packing and assembly at the interface. The newly synthetic amphiphilic lipids displayed excellent dual functionality as oil-in-water emulsifiers and as antioxidants against lipid peroxidation.

Natural vs. Synthetic Phospholipids: Pharmaceutical Applications as Drug Delivery System. Moghis Ahmad*, Shoukath M. Ali, Ateeq Ahmad, Saifuddin Sheikh, Paul Chen, and Imran Ahmad, *Jina Pharmaceuticals Inc., USA*

Phospholipids have been extensively studied as drug delivery vehicles and continue to create interest in this field. Naturally occurring phospholipids are isolated from egg yolk, bovine-derived, soybean, sunflower and canola seeds. In some instance, these phospholipids are converted to saturated phospholipids by hydrogenation or treated with enzymes such as phospholipase A2 to partially remove fatty acids or phospholipase D to convert polar head group. These modified natural phospholipids (saturated and/or unsaturated) are also considered as natural phospholipids because the resulting phospholipids occur in nature. The use of egg or bovine-derived phospholipids are generally restricted in clinical applications due to the possibility of viral or protein contamination. Phospholipids where polar head groups or fatty acids are introduced by means of chemical synthetic process are known as synthetic phospholipids. While natural lipids should be selected for formulation development wherever possible, the clinical advances would not have been possible without the development of synthetic lipids. Depending on the nature of the drug, naturally occurring and/or synthetic phospholipids can be used to develop drug delivery vehicles. This approach has paved the way for the clinical and commercial success to entrap both hydrophobic and hydrophilic drugs to significantly reduce its toxicity. The development of novel synthetic lipids for drug delivery to reduce toxicity and improve therapeutic efficacy of a drug will be discussed.

Novel Syntheses of Phospholipids. Chris Dayton*, *Bunge Limited, USA*

Phospholipids are a major component of all cell membranes. They form the bilayer of the plasma allowing them to be selectively permeable to proteins, ions and water. Phospholipids are amphiphilic, describing a chemical compound possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties. The amphiphilic nature of phospholipids can be modified and improved by various chemical and enzymatic reactions. An overview of these reactions and the functionality of these new molecules will be discussed.

PHO 3: Bioactive Phospholipids and Lipids for Drug Delivery

Chair: Ernesto Hernandez, Advanced Lipid Consultants, USA

Effects of A Purified, Omega-3 Rich Krill Oil Phospholipid on Cardiovascular Disease Risk Factors and Fatty Acid Composition of Erythrocyte Membranes Nils Hoem*, *Aker Biomarine Antarctic AS, Norway*

Omega-3 fatty acids in krill oil are mostly associated with phospholipids (PL) of which phosphatidylcholine is the dominant species. Normal krill oil consists of approx. 40% PL while the rest is mostly triglycerides, We purified the PL fraction of krill oil up to approx. 98%, formulated it for oral administration using PEG400 and ethanol as fluidizers and administered the resulting preparation to 3 type-2 diabetic, dyslipidemic non-human primates (NHPs) at escalating doses for a total of 12 weeks. A control group of 3 NHPs was given the same vehicles in water. PL doses used were 50, 150 and 450 mg/kg/day and each dose-level was given for 4 weeks. Corresponding EPA/DHA doses were 9.4/5.5, 28/16.5 and 84/49 mg/kg/day, respectively. Blood samples were taken weekly for analysis of cardiovascular disease (CVD) risk factors (cholesterols, apolipoproteins and triglycerides), bi-weekly for diabetic parameters (Hb1Ac, glucose and insulin) and for determination of erythrocyte concentrations of 22 other fatty acids, including the omega-3 index, and every 4 weeks for safety parameters and pharmacokinetics. Upon cessation of dosing, the same parameters were followed for an additional 8 week wash-out period. During the 4 weeks with the lowest dose, no marked effects were observed while when the intermediate and high doses were given, total cholesterol, LDL and triglycerides were significantly reduced while HDL increased. During wash-out, all parameters reverted back towards the baseline values. The omega-3 index increased from approx. 6% at baseline up to 18% at the end of week 12. Saturated fatty acids in membranes, whether abundant or not, were unaffected by the increase in omega-3 index, the same was true for trans-fatty acids. The only fatty acids changing markedly were omega-6 fatty acids, e.g. arachidonic acid was down from about 12% to about 8%. The largest change was seen for di-homo-g-linolenic and docosatetraenoic acid, both were reduced by approx. 70% compared to baseline. It is concluded that the purified, omega-3 rich PL derived from krill oil had beneficial effects on several CVD risk factors and changed the cell membrane fatty acid composition markedly, resulting in a clearly altered omega-6/omega-3 ratio.

"L3, Lipids-based Sponge Phases"—Characteristics and Uses as Drug Carriers Yosef Brody and Nissim Garti* *Casali Institute of Applied Chemistry The Hebrew University of Jerusalem, Israel*

Many different products are available today for treatment of various skin disorders, such as Acne and Psoriasis. One such class is antibiotics, which target the bacteria involved in causing these disorders. The main drawback of this treatment type lies in that the antibiotics are administered orally, which leads to low availability and also can cause many adverse effects. The advantages of a topical antibiotic which could be administered directly to the affected area are clear, however very few such products exist, mainly due to the low solubility of these antibiotics in water, and also due to their instability in water. The antibiotic Minocycline HCl is a drug used for the treatment of acne, yet as of today it is administered orally. The focal point of this work is the attempt to create a formulation for the topical delivery of Minocycline HCl, using a little-researched mesophase coined L3 or 'sponge phase'. This mesophase is comprised of intertwined aqueous channels separated by a hydrocarbon chain bilayer. The existence of this phase was first reported in 19801, but its full potential as a drug carrier has yet to be fully realized. This presentation will discuss different

physicochemical aspects related to the characterization of this mesophase such as the structure, dynamics, and location of the drug and other components within the system by means of several analytical methods such as SAXS, Rheology, Self-Diffusion NMR and various other methods.

Fucoxanthin Improves HbA1c in G/G Allele Carriers of UCP1 Polymorphism in Japanese: Rumoi Fucoxanthin Study Nana Mikami*[1], Masashi Hosokawa[2], Kazuo Miyashita[2], Hitoshi Sohma[1], Yoichi M. Ito[3], and Yasuo Kokai[1], *[1]School of Medicine, Sapporo Medical University, Japan; [2]Hokkaido University, Japan; [3]Dept. of Biostatistics, Hokkaido University Graduate School of Medicine, Japan*

Fucoxanthin (Fx) is a marine carotenoid found in brown algae and known to possess health beneficial effects in vitro and in vivo. We previously performed Fx intervention trial onJapanese adults and observed improvement of HbA1c level, a marker reflecting glycemic control. To further investigate its effectiveness, we analyzed HbA1c variation by Fx intake based on genetic factor, in particular, UCP1 gene polymorphism. The study design was a single-blinded and randomized controlled trial. Sixty healthy adult participants with BMI ≧ 22 were assigned to three groups supplemented each capsules (containing 0, 1, or 2 mg Fx) every day for eight weeks. They were genotyped for -3826 A/G polymorphism of UCP1 gene and stratified to A/A or A/G or G/G alleles. Considering the polymorphism, allele frequencies were not different between three groups. The variation of HbA1c was significantly lowered in Fx 2 mg group compared to 0 mg group. Interestingly, in 2 mg group, the variation in participants with G/G allele decreased significantly greater than those with A/A and A/G alleles. Our results indicate that UCP1 gene polymorphism may influence Fx effectiveness on HbA1c levels. This is the first report to evaluate health beneficial effect by Fx intake with nutrigenetic approach.

Synthesis a Novel Biopolymer for Drug Delivery Nisarg K. Prajapati*[1], and Nirmal K. Patel[2], *[1]V. P. & R. P. T. P. Science College, India; [2]Institute of Science & Technology for Advanced Studies & Research, India*

In present work on novel starch based biopolymer preparation. Biopolymers are advanced polymer systems that hold special advantages for the delivery of drugs and enhancing the biocompatibility of implantable devices. For the treatment of many diseases large molecular weight proteins are required. These can be available with the availability of Hydrogels. Hydrogels are hydrophilic, three-dimensional networks, which are able to imbibe large amounts of water or biological fluids, and thus resemble, to a large extent, a biological tissue. First, extract starch from the potato and purification and etherification of starch by using mono chloropropionic acid. Improve control drug release property of etherified starch prepared new high water holding, biocompatible and biodegradable monomer 2,3-dihydroxypropyl acrylate monomer grafted with etherified starch prepared polymer matrix (biopolymer). The resulting biopolymers use as a drug carrier with loading a specific drug. The conform monomer structure using ^{1}3C NMR. The structure of biopolymer conformed to using SEM and FTIR. The study of its drug release property and compeer drug release activity of polymeric drug and standard drug.

Excipient Emulsions Design: Enhancing Nutraceutical Bioavailability from Natural Foods Ruojie Zhang*, Zipei Zhang, and David J. McClements, *University of Massachusetts Amherst, USA*

Daily diet is the most important source of nutraceuticals (bioactive components in foods that improve health and wellbeing) for humans. However, the bioavailability of nutraceuticals from natural foods is often very low and variable, especially for highly hydrophobic nutraceuticals, such as carotenoids. Therefore, there is a need to

design approaches to efficiently enhance the bioavailability of nutraceuticals from natural foods so as to improve human health and wellbeing. Novel excipient emulsions were specifically designed to enhance the bioavailability of these lipophilic nutraceuticals. The composition and microstructure of the excipient emulsions were specifically design to enhance hydrophobic nutraceuticals. Simulated gastrointestinal tract including mouth, stomach and small intestine was used to study the efficacy of excipient emulsion at controlling hydrophobic nutraceutical release, solubilization, transport, metabolism, and absorption within the gastrointestinal tract. In addition, the possible side effects of excipient emulsion for human health (such as increasing the bioavailability of hydrophobic pesticides) were also studied. This knowledge could be used to develop a new range of excipient food products specifically designed to increase the bioavailability of bioactive agents from natural products (such as fruits, vegetables, cereals, meats, or fish), e.g., excipient sauces, dressings, dips, creams, or beverages.

Role of Phospholipids in Delivery and Stabilization of Omega-3 Fatty acids in Nanoemulsions Ernesto Hernandez*, *Advanced Lipid Consultants, USA*

Abstract not available.

PHO-P: Phospholipid Poster Session

Hydrolysis of Epoxyeicosatrienoic and Epoxydocosapentaenoic Acid Esters of Glycerophospholipids by Group IIA, V, and X Secretory Phospholipases A2 Arnis Kuksis*, and Waldemar Pruzanski,*University of Toronto, Canada*

Cytochrome P450 epoxygenases convert arachidonic to epoxyeicosatrienoic acid (EET), and docohexaenoic to epoxydocosapentaenoic acid (EDP) . Both free and esterified epoxy fatty acids are also formed during lipid peroxidation. For biological activity the phospholipid epoxides require hydrolysis, which we have investigated using peripheral human Group IIA, V and X sPLA2 using autooxidized plasma lipoproteins as epoxide substrates. The lipoproteins were isolated by ultra centrifugation and were analyzed by LC/ESI-MS, as previously described (Pruzanski et al, 2000). The monoepoxides were identified by reference to standards and to retention times of phospholipid masses extracted by computer. For quantification of phospholipid epoxides we used endogenous n18:1/16:0 PCho as internal standard estimated at 90, 20, and 7 nmoles/mg protein of LDL, HDL and HDL3, respectively. The PtdCho monoepoxides in stored human LDL ranged from 0-5 nmol/mg protein, which increased to 5-10 nmol/mg protein following incubation for 4 hours at 37 oC. A 4h incubation of autoxidized LDL or HDL with 1µg/ml of group V and X sPLA2 resulted in a 40-50% hydrolysis, while group IIA sPLA2 at 1 µg/ml failed to produce significant hydrolysis. With 2.5 µg/ml of sPLA2s, there was nearly complete hydrolysis of epoxide ester with group V and X sPLA2 in 1h, while group IIA sPLA2 yielded 20% hydrolysis in 4h. The hydrolysis was complete in 8-24h. EET are proangiogenic and have been shown to accelerate tumor growth and metastasis by stimulation of tumor angiogensis, while EDP inhibit angiogenesis, tumor growth and metastasis (Zhang et al., 2013).