AFTERNOON

PHO 1: Lecithin from Alternative Sources

Chair(s): M. Tomás, CIDCA, Argentina; and B. Sebree, Archer Daniels Midland Co., USA

Fractionation of Sunflower Lecithin at different processing conditions. E.N. Guiotto[1], D.M. Cabezas[1], B.W.K. Diehl[2], M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos, CONICET, Facultad de Ciencias Exactas (UNLP), La Plata, Buenos Aires, Argentina, [2]Spectral Service GmbH, Cologne, Germany

Sunflower lecithin is an important by-product in countries producing important amounts of crude sunflower oil. Due to its high phospholipids and essential fatty acid contents, it can be well used as an additive in foods. A fractionation process of sunflower lecithin with different ethanol/water mixtures was carried out for obtaining enriched fractions in phosphatidylcholine (PC) and phosphatidylinositol (PI). The effect of ethanol/water composition (90:10 to 96:4) and pH (1.2 to 10.5) on the extraction efficiency was studied. Phospholipid enrichment and the composition of PC and PI fractions were determined by yield% and 31PNMR. The percent extraction coefficients for different phospholipids (%EPC, %EPE and %EPI) in both fractions were calculated. The increase of ethanol content and pH level produced the highest enriched PC fraction yield, %EPC and %EPE with mixtures 96:4 which increased significantly from 49.2 and 18.3% (pH 1.2) to 56.4 and 24.8% (pH 10.5), respectively. A high water content in the ethanolic mixture (90:10) resulted in a considerable decrease in PC and PE extraction; %EPC and %EPE values varied from 39.3 and 11.3% (pH 1.2) to 48.2 and 15.2% (pH 9.2), respectively. %EPI values (< 4%) showed the high insolubility of PI.

Elaboration and Characterization of Nanoliposome Made of Soya, Rapeseed and Salmon Lecithins: Application to Cell Culture. Elmira Arab Tehrany[1], Cyril Kahn[2,3], Christophe Baravian[2], Behnoush Maherani[1], Nabila Belhaj[1], Xiong Wang[2], Michel Linder[1], [1]Nancy-université,LiBio, Nancy, France, [2]Nancy-université, Lemta, Nancy, France, [3]Nancy-université, LPPIA, Nancy, France

Health benefits of unsaturated fatty acids have been demonstrated over the last decades. Nanotechnology provided new process to produce particles such as liposomes and nanoliposomes made of pure phospholipids. These techniques are already used in pharmaceutics to augment the bioavailability and the bioefficiency of drugs. The aim of this paper is to characterize and evaluate the potential of nanoliposomes made of three lecithins (soya, rapeseed and salmon) on cell culture in order to use them in the future as drug delivery systems for tissue engineering. We began to measure, with zetasizer, the radius size of liposomes particles which are 125.5, 136.7 and 130.3 nm respectively for rapeseed, soya and salmon lecithin. Simultaneously, solutions observed by TEM demonstrated the particles were made much of liposomes than droplet (emulsion). Finally, we found that the solutions of lecithins were enough stable over 5 days at 37Â°C to be used in culture medium. We investigated the effect of soya, rapeseed and salmon lecithin liposome from 2 mg/mL to 5.2 Âµg/mL on metabolic activity and cell proliferation on rat bone marrow stem cells (rBMSC) during 14 days. The results showed that the three lecithins (soya, rapeseed and salmon) improve cell proliferation at different concentration.

Oxidative Stability of Marine Phospholipids Emulsions. Henna Fung Sieng Lu, Caroline Baron, Nina Skall Nielsen, Charlotte Jacobsen, Technical University of Denmark, Denmark

Many studies have shown that marine phospholipids (MPL) provide more advantages than fish oil. The objective of

this study is to investigate the oxidative stability of emulsified MPL, which also includes the non-enzymatic browning reaction. This study also investigates the effect of chemical composition of marine PL towards their stability. Firstly, emulsions were prepared by high pressure homogenizer with different types and levels of MPL. In some formulations, fish oil was also added in order to study the effect of increasing levels of triglycerides in the emulsions. Then, the oxidative and hydrolytic stability of emulsions was investigated by measurement of Peroxide Value and Free Fatty Acids, and 31PNMR after 32 days storage at 2ºC. The oxidative stability of MPL emulsions was further investigated through 1HNMR and measurement of secondary volatile compounds by Solid Phase Microextraction GC-MS at several time intervals. In addition, the non-enzymatic browning reaction was investigated through the measurement of color changes and pyrroles content. The obtained result suggested that MPL emulsions have relatively good oxidative stability as compared to fish oil containing emulsions. As a conclusion, MPL with different chemical compositions have affected emulsions stability differently.

Synergy in the use of Phospholipases for Degumming of Vegetable Oils. Chris Dayton, Flavio Galhardo, Bunge Global Innovation, USA

Enzymatic degumming for removal of phospholipids in vegetable oil sources has grown dramatically over the years. The original focus of using enzymes was to aid in the removal of the so-called "non-hydratable" phospholipids without the need of large amounts of acids. Today, the focus is on yield improvements and environmentally friendly processing.This presentation will focus on the synergist effects of using multiple enzymes in the removal of phospholipids from oils thereby further increasing the yields, but also the unique biochemistry involved.

Emulsifying Properties of Hydrolized Sunflower Lecithins by Phospholipases A_2 of Different Sources. D.M. Cabezas[1], R. Madoery[2], B.W.K. Diehl[3], M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA?CONICET-UNLP), 47 y 116 (1900), La Plata, Argentina, [2]Cátedra de Química Orgánica ? Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba (FCA - UNC), Ciudad Universitaria S/N (5000), Córdoba, Argentina, [3]Spectral Service GmbH Laboratorium füür Auftragsanalytik, Emil Hoffman Str. 33, D-50996 Cologne, Germany

Sunflower (Helianthus annuus L.) lecithins are obtained by gum purification from raw oil by a degumming process. Food industry uses lecithins because of their multifunctional ingredients. Modification processes such as enzymatic hydrolysis, are appropriate for certain applications. The aim of this work was to evaluate the emulsifying properties of different modified sunflower lecithins in O/W emulsions. In this study, hydrolyzed sunflower lecithins (HSL), which were obtained by enzymatic hydrolysis with PLA_2 from pancreatic porcine and microbial sources, were assessed using a deoiled lecithin (DSL) such as a control system. This modified lecithins were applied as emulsifying agent in O/W emulsions (30:70 wt/wt), ranging 0.1?2.0% (wt/wt). Stability of different emulsions was evaluated through the evolution of backscattering profiles (%BS), particle size distribution, and mean particle diameters. HSL presented the best emulsifying properties against the main destabilization processes for the emulsions studied in comparison with DSL. These modified lecithins represent a good alternative for the production of new bioactive agents. Furthermore, the use of a microbial phospholipase gives the possibility to obtain a spectrum of sunflower lecithins which functionality could be applied to the development of kosher and halal foods.

Technology of Dry Powder Sunflower Lecithin for Food, Feed and Pharmaceuticals. Sergiy M. Shulga, Igor S. Glukh, Institute for Food Biotechnology and Genomics, Kyiv, Ukraine

Currently, commercially available phosphatidylcholine is obtained from soya beans and egg yolks. Use of the sunflower phosphatide concentrate as source material is one of promising trends for the commercial use of phosphatidylcholine. However, the complexity of the matrix requires specific approaches for its effective extraction and purification.A method is worked out for solid-phase extraction, concentration and purification of phosphatidylcholine using silica gel and aluminum oxide as sorbents for the separation of the components of sunflower lecithin and purification of phosphatidylcholine.The task of the proposed useful (utility) model is to improve the fraction composition of lecithin when using food phosphatidic concentrates as raw material, and to make possible the use of feed phosphatidic concentrates as raw materials for lecithin obtaining. The use of the proposed method allows to get lecithin without impurities, which is in line with best world analogues.The essence of the proposed method is to

determine the technological parameters of phosphatidic concentrate degreasing by its extraction by acetone.The technology is developed and industrial lots of dry clean powder phosphatidylcholine (L-lecithin) are obtained.

GumZyme^{TM} Phospholipase A2 has Preference for Phosphatidic Acid, Ideal for Enzymatic Oil Degumming. A. Sein, H.M.W.J.C. Uijen, A. de Roos, W. Smits, DSM Food Specialties, Delft, the Netherlands

DSM is introducing *GumZyme*^{TM}, a phospholipase A2 (PLA2) to improve the oil degumming process. This PLA2 catalyzes the hydrolysis of phospholipids into lysophospholipids and fatty acids. In the pool of phospholipids found in crude seed oils, this PLA2 shows a preference for the poorly hydratable ones, phosphatidic acid (PA) and phosphatidylethanolamine (PE). Since PA and PE are difficult to remove during the degumming process, the preference of this PLA2 for these compounds makes gum removal in the presence of this PLA2 more efficient. It will lead to higher oil yields in degumming of crude oil by more efficient phase separation, and to more efficient removal of remaining poorly hydratable phospholipids from water degummed oil in the deep-degumming process. The enzyme works efficiently at fairly neutral pH conditions and up to 70°C/160°F, and has no lipase side activity. It is produced by microbial fermentation of a selected strain of *Aspergillus niger* and is Kosher and Halal certified.

TUESDAY

MORNING

PHO 2: Structured Phospholipids and Lysophospholipids

Chair(s): X. Xu, University of Aarhus, Denmark; G. Wang, Cargill, USA; and M. Ahmad, Jina Pharmaceuticals, Inc., USA

Lysophospholipids as biomarkers of oxidative stress. Beate Fuchs, Celestina Schober, Gerrit Vortmeier, Xiyu Ouyang, Jürgen Schiller, University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Leipzig, Saxony, Germany

Inflammatory diseases are associated with pronounced oxidative stress. At inflammatory loci, hypochlorous acid (HOCl) is generated by myeloperoxidase in significant amounts. HOCl reacts with a variety of biomolecules and induces the generation of lysophosphatidylcholine (LPC) from polyunsaturated phosphatidylcholines (PC).As many tissues and cells contain huge amounts of polyunsaturated PC species enhanced LPC concentrations are normally detectable under inflammatory conditions. However, human (e.g. liver) samples contain also major amounts of polyunsaturated phosphatidylethanolamine (PE). Unfortunately, it is so far widely unknown, whether PE oxidation leads to LPE generation in the same manner as LPC is derived from PC. In the extracts of liver biopsies from patients suffering from inflammatory diseases LPC and LPE formation could be detected by ^{31}P NMR spectroscopy and MALDI-TOF MS. Both lysophospholipids (LPL) are assumed to be massively generated under the influence of the enzyme phospholipase A_2 (PLA_2) although previous work provided evidence that LPC is also generated by HOCl alone. We have shown by MALDI-TOF MS and ^{31}P NMR that PC - but not PE - is characterized by subsequent LPL formation upon treatment with HOCl. Summarizing, LPE is a reliable *in vivo* biomarker of PLA_2 activity, whereas LPC may be generated by PLA_2 and HOCl.

Analysis of Lysophospholipids and Glycerophosphates (double lyso) by ^{31}P-NMR. B. Diehl, Spectral Service GmbH, Germany

Lysophospholipids can be designated products prepared from phospholipids of many different sources as well as undesired degradation products in raw material or finished products. In both cases a proper analysis is necessary to obtain accurate values of these phospholipid species. The paper presents studies for monitoring the enzymatically preparation of defined lysolecithins and storage stability studies of phospholipids in food and drugs.

Structured Phospholipids: Synthesis and Applications in Food Systems. Ling-Zhi Cheong, Xuebing Xu, Department of Engineering, Aarhus University, Aarhus C, Denmark

Phospholipids (PLs) are amphiphilic molecules containing both hydrophilic head group and hydrophobic tail. Due to its amphiphilicity, PLs can be used as emulsifiers to stabilize various food emulsion systems. In addition, PLs self-assemble into various supramolecular structures such as bilayer, capsule and liposome. These structures specifically liposome can be used to encapsulate active ingredients for enhanced delivery and bioavailability. Structural modification of PLs especially through enzymatic means has been done quite intensively to produce structured PLs with more diverse applications. The enzymatic modification can be either modifying the fatty acid chain or hydrophilic head group. A review on current state of the art in enzymatic modification of PLs will be given with emphasis on the critical factors affecting the overall reaction rate. In addition, the physicochemical properties and possible application of these structured PLs in food system will also be discussed.

Diversity of Phospholipid Effect on Oil Oxidation. O.T. Kasaikina, E.A. Mengele, D.A. Krugovov, L.M. Pisarenko, N.N.Semenov Institute of Chemical Physics RAS

Phospholipids are widely used in food, cosmetic and drug production. The effect of egg lecithin (Fluka) (PL) on methyl linoleate, limonene, and palm oil oxidation by oxygen under various conditions (initiated, inhibited, and catalyzed by transient metal oxidation) was studied. Lecithin was found to form mixed micelles together with amphiphilic lipid hydroperoxides (LOOH). Because polar phenolic antioxidants concentrate in mixed micelles, the escape of free radicals derived from thermal decomposition of LOOH can be decreased noticeably. It results in the decrease of initiation and consequently oxidation rates. The rate of chain transfer by inhibitor's radical decreases in reverse micelles as well. So, phospholipids demonstrate synergism in prevention of oxidation with polar phenols. In some cases, lecithin was shown to trap transient metal compound inside micelles and prevent oxidation of bulk oil.Computer simulation of the cooxidation of PL with methyllinoleate and limonene revealed a key role of radical cross-propagation and cross-disproportionation reactions which determined the common oxidation rate.

AFTERNOON

PHO 3: General Phospholipids

Chair(s): B. Sebree, Archer Daniels Midland Co., USA

Effect of Calcein on Model Lipid Membranes. Behnoush Maherani[1], Elmira Arab-tehrany[1], Beata Korchowiec[2,3], Ewa Rogalska[3], Michel Linder[1], [1]Institut National Polytechnique de Lorraine, Nancy, Lorraine, France, [2]Jagiellonian University, Cracow, Poland, [3]CNRS/Nancy-Université, Nancy, Lorraine, France

Liposomes commonly used as a controlled release carrier. One of the noticeable factors in release profiles is the strength of drug-carrier interaction.To adjust the pharmacokinetic and pharmacodynamic properties of therapeutic agents, optimizing the drug-carrier interaction is necessary.To get a better understanding of such interaction, large unilamellar liposomes containing calcein were prepared using DOPC,POPC,DPPC, and their mixture; calcein was chosen as a model polar molecule.The thermodynamic changes caused by calcein and its location into lipid bilayers

were determined by Differential Scanning Calorimetry and Raman spectroscopy.The results reveal that calcein influences the thermotropic properties of lipid membrane causing slightly decrease in phase-transition. The intensity changes of Raman peaks represent the interaction of calcein with choline head groups.Moreover, the impact of calcein on phosphoglyceride Langmuir layers spread at the air-water interface was studied using surface pressure-area and surface potential-area isotherms, as well as polarization-modulation infrared reflection-absorption spectroscopy and Brewster angle microscopy. The results indicate that while calcein has a meaningful effect on the systems prepared with pure lipids, it is attenuated in the case of the lipid mixtures. Our observations may be useful for developing efficient liposomal systems in delivering polar drugs.

Normalization of Phospholipid Membrane Structure with Phospholipid Emulsion in Neurological Disease. P.C. Kane, M.O. Speight, S. Pouria, K. Bieber, J. McLaren-Howard, NeuroLipid Research Foundation, Millville, NJ USA

The lipid soluble nature of toxins has led us to address the complexity of neurotoxicity oriented to cell membrane architecture. Identification of nuclear and mitochondrial DNA adducts as microbials, chemicals, pesticides, and metals at Acumen Laboratory in Devon, England and red cell lipid analysis at Johns Hopkins, Peroxisomal Diseases Laboratory were obtained on subjects with MS, Autism, Post Stroke, Alzheimers, ALS, Neurometabolic disorders and Parkinsons. Our previous findings revealed a link between toxic exposures, a characteristic accumulation of very long chain fatty acids (VLCFAs) in the form of lipid rafts or ceramides, and the development of cell membrane derangement resulting in dysfunction. In our current study we have captured visual images of toxins on the cell surface which have caused disturbances in cellular phospholipid structure of our subjects, linking the impact of the DNA adducts (toxins) altering gene expression to aberrations in lipid metabolis, cellular dysfunction and alteration of the structure of phospholipids in the cell membrane characteristic to the presenting diagnosis and symptoms. Our treatment protocol of has yielded significant clinical neurological improvement in our subjects corresponding with normalization of cellular structure via images of the subjects membrane phospholipids.

WEDNESDAY

N/A