106th AOCS Annual Meeting and Industry Showcases

Phospholipid Division Technical Program Abstracts

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The presenter is the first author or otherwise indicated with an asterisk (*).

PHO 1: General Phospholipid

Chair: M. Rebmann, Perimondo, USA

The Effect of Microfiltration on Milk Fat Globule Distribution, Yield, and Physical Properties of Cheddar Cheese. A. Logan, M. Xu, M. Mazzonetto, L. Day, and M.A. Augustin, CSIRO Food and Nutrition, Australia.

The benefits of controlling the milk fat globule (MFG) size distribution of raw milk for Cheddar-cheese production was examined for cheese yeild, composition and texture over 6-months of maturation at 8°C. Cheddar cheese was produced from milk fractionated on the basis of MFG size using a two-step microfiltration process to produce small (~3um) and large (~5um) MFG milk fractions. Yield gains were achieved in cheese made from small MFG milk compared with both the Control and large MFG milk. Further, confocal laser scanning microscopy revealed differences in microstructure, specifically the size of fat pockets and number of intact smaller MFG embedded within the protein network structure. Cheese produced from small MFG milk contained more total polar lipids in comparison. Changes over time in texture, including cheese hardness, cohesiveness and springiness, were consistent with expectations for matured cheese. However textural differences between samples indicate microfiltration for MFG size may affect consumer perceptions and could reduce the time required for Cheddar cheese to reach markers associated with cheese maturation.

Effect of Phospholipid and Other Factors on Free Radicals Escape in Hydroperoxide Decomposition Catalyzed by Acetylcholine. O.T. Kasaikina, D.A. Krugovov, and E.A. Mengele, Russian Academy of Sciences, Russia.

Acethylcholine chloride (ACh) has been found to catalyze both lipid hydroperoxide (LOOH) and hydrogen peroxide (H_2O_2) decomposition into free radicals in organic media. Because LOOH are the primary lipid oxidation products, ACh accelerates lipid oxidation by oxygen. By means of inhibitor's method using different radical acceptors, free radical initiation (R_i) rates were measured. It was found that phospholipid (egg lecithin, PCh) decreased the escape of free radicals derived from decomposition of LOOH catalyzed by ACh. DLS method was used for measuring the size and size distribution of microaggregates {xACh.. yLOOH} and effect of PhC on their sizes. The increase of sizes in the presence of PhC gives an evidence of solubilization of polar microaggregates {xACh.. yLOOH} by PCh and preventing thereby free radical escape into bulk solution.

Free radical initiation in the system: ACh - tert-butyl hydroperoxide in n-decane at 37°C was studied in detail. It was found that R_i increases under hypoxia condition that is oxygen in some extent decreases free radical escape into bulk solution. Moderate magnetic field decreases R_i in this system as well, especially in the absence of O_2.

Influence of Curcumin-loaded Chitosan Liposome on MCF7 Cytotoxicity. M. Hasan[1], E. Arab-Tehrany[1], M. Barberi-Heyob[1], C. Kahn[2], M. Linder[1], and E. Jabbari[3], [1]Université de Lorraine, France, [2]IFSTTAR, France, [3]University of South Carolina, USA.

The role of curcumin (diferuloylmethane), for cancer treatment has been an area of growing interest. However, due to its low absorption, the poor bioavailability of curcumin limits its clinical use. In this study, we encapsulate the curcumin in chitosan loaded liposome from natural composition (salmon, rapeseed and soya lecithin) in order to increase the solubility and bioavailability of curcumin. The physic-chemical properties of liposome, chitosan-coated liposome and with and without curcumin were studied. The membrane fluidity, size, potential zeta and morphological properties were measured in order to understand the influence of chitosan on structural properties. In addition the in vitro test was realized to study the cytotoxicity of vector with and without active molecule. We observed a high loading efficiency of curcumin in chitosan-coated liposomes and strong cytotoxicity towards MCF7 cells. Among the three lecithins, the salmon lecithin has an effect important on MCF7 cytotoxicity. The presence of various polyunsaturated fatty acids in salmon lecithin improve its efficiency.

Development and Modernization of USP-NF Public Standards for Phospholipids. H. Wang, US Pharmacopeial Convention, USA.

On December 15, 1820, the first edition of *The Pharmacopoeia of the United States* was published. Over time, the nature of the *United States Pharmacopeia* (*USP*) changed from being a compendium of recipes to a compendium of

documentary standards for identity and quality that typically involve reference materials used as comparison standards in specified tests and assays. This presentation introduces the development and modernization of the United States Pharmacopeia–National Formulary (USP-NF) phospholipid excipient monographs. Phospholipids are processed from natural sources such as egg or soybean oil, and have complex chemical compositions. As part of the public standards-setting processes, USP staff and the Excipients Expert Committee have developed a strategic analytical testing plan for phospholipid excipients. With a depth of research studies for those naturally derived materials and an evolvement of analytical technology, monograph development and modernization are able to introduce more specific compositional methods that can identify as well as quantify the analyte(s) of interest. A combination of orthogonal methods uses existing instrumentation that can be applied to a multitude of phospholipid monographs and encourages ease of adoption. This plan also has helped make possible the development of USP Reference Standards for such complex excipients because the proposed analytical methods provide a comprehensive understanding and characterization of phospholipids.

PHO 2: Nutrition and Analytics of Phospholipids

Chair: B.W.K. Diehl, Spectral Service AG, Germany

Emulsifying Properties of Hydrolyzed and Low HLB Modified Sunflower Lecithin Mixtures. D.M. Cabezas[1], B.W.K. Diehl[2], and M.C. Tomás*[3], [1]Universidad Nacional de Quilmes, Argentina, [2]Spectral Service AG, Germany, [3]Universidad Nacional de La Plata, Argentina.

Most commonly processes for lecithin modification are deoling, fractionation with absolute ethanol to produce PC or PI enriched fractions or enzymatic hydrolysis of the starting material. These modifications are important for achieving an optimal balance between hydrophilic and lipophilic compounds (HLB) and ensure a good food processing ability. The aim of this work was to evaluate the capacity of a sunflower hydrolyzed lecithin to influence the performance of modified lecithins with low HLB as emulsifier. Thereby, sunflower hydrolyzed lecithin (SHL) was obtained by enzymatic hydrolysis with a microbial PLA_2 and mixed with a deoiled (DSL) or a PI fraction (PIf) from the same source. These modified lecithin and their mixtures were applied as emulsifying agent of coarse and fine O/W emulsions (F_m=0.3). Stability of different emulsions was evaluated through the evolution of backscattering profiles, particle size distribution, and mean particle diameters. SHL increased the HLB (addition of lysophospholipids) and improved the emulsifying properties of DSL and PIf ineach case. Mixtures 50:50 of SHL- DSL or SHL-PIf produced a similar behavior than 100% SHL emulsions constituing a good alternative for the production of new bioactive agents.

Detection of Additives and Contaminations of Sunflower Lecithin with Soy Bean Lecithin. B.W.K. Diehl[1] and K. Rizos[2], [1]Spectral Service AG, Germany, [2]Genetic ID (Europe) GmbH, Germany.

Differentiation of sun flower and soy bean oil is an important analytical challenge. Due to different acceptance of GMO soy bean lecithin the purity of alternatives from sunflower based lecithin must be analyzed by valid methods. A differentiation between active blending (5/ to 95%) and accidental contamination < 2% and vice versa must been detectable and in a certain limit quantifiable. Combinations of NMR and PCR investigations gives the analytical solution for these problems.

Lecitase Ultra-catalyzed Hydrolysis of Soy Phosphatidylcholine to Prepare LPC and L-a-GPC. B.H. Kim, Chung-Ang University, Republic of Korea.

The aims of this study were to model Lecitase Ultra-catalyzed hydrolysis of soy phosphatidylcholine (PC) in hexane-water media for the production of LPC and L-α-GPC, respectively, and to optimize the reaction conditions, respectively, using response surface methodology. The reactions were performed in a stirred batch reactor. The effects of temperature (Te), reaction time (RT), water content (WC), and enzyme loading (En) on LPC and L-α-GPC content in the reaction products were elucidated using the models established. Optimal conditions for maximizing the LPC content while suppressing acyl migration, which causes L-α-GPC formation, were as follows: Te, 60°C; RT, 3 h; WC, 10% of PC; and En, 1% of PC. The products obtained under these conditions contained 16.3% PC, 55.9% LPC, 27.8% free fatty acid (FFA), and no GPC. While, optimal conditions to completely hydrolyze PC to L-α-GPC were: Te, 50°C; RT, 30 h; WC, 69% of PC; and En, 13% of PC. The products obtained under these conditions contained 21.0% L-α-GPC, 71.1% FFA, and 7.9% phosphocholine, but were free of PC and LPC. The FFA and phosphocholine were completely removed using a solvent extraction procedure and a silica column chromatography, respectively.

^{1}H NMR as Release Analysis for Infant Nutrition. B.W.K. Diehl, M. Betzgen, and Y. Monakhova, Spectral Service AG, Germany.

NMR spectroscopy is developed and validated as a powerful tool for release analysis of infant nutrition. The method allows the simultaneously detection and quantification of several lipids and carbohydrates as well as other active ingredients. A single analysis of organic and water soluble fractions gives quantitative values for TAG, PC, SPH, PE, cholesterol, sitosterol, fatty acid composition, omega-3 FA (vegetable oil), butyric acid (milk), CLA, lactose, sucrose, glucose, fructose and oligosaccharide (e.g. LNT), calcium, magnesium, zink, citric acid, free amino acids, lactic acid and potential other additives or frauds.

By principle component analysis (PCA) even the type of the formula and the manufacturer of the products are veritable.

Determination of Phospholipids in Olive Oil Using Multinuclear NMR Spectroscopy. E. Hatzakis, Pennsylvania State University, USA.

Abstract not available.

PHO-P: Phospholipid Poster Session

Chair: B. Sebree, Archer Daniels Midland Co., USA

1. **Synthesis and Characterization of New Carbohydrate-lipid Conjugates for Drug Targeting.** M.U. Ahmad[1], S.M. Ali[1], A. Ahmad[1], S. Sheikh[1], P. Chen[2], and I. Ahmad[1], [1]Jina Pharmaceuticals, Inc., USA, [2]Nia Life Sciences, USA.

A new synthetic methodology for cationic glycolipids using p-aminophenyl-a-D-mannopyranoside (PAPM) and p-aminophenyl- a-D-galactopyranoside (PAPG) was developed. A spacer was introduced between the quaternary nitrogen atom and the sugar unit. In addition, a new class of neutral glycolipid conjugates, such as PAPM-lipids or PAPG-lipids conjugates was also synthesized for targeting of drugs to specific carbohydrate receptors. The precipitation-inhibition assay showed that conjugate of PAPM inhibited the interaction between Concanavalin A and Invertase, a mannose-containing glycoprotein. These new lipid-based molecules can be effectively utilized using nanosomal or liposomal drug delivery system for site specific delivery of drugs. The synthesis of the lipid-carbohydrate conjugates and the binding inhibition studies will be the subject of discussion.

2. **Individual Phosphatidylcholine Species Analysis by RP-HPLC-ELSD for Determination of Polyenylphosphatidylcholine in Lecithins.** W.J. Lee and N.W. Su, National Taiwan University, Taiwan.

This study aimed to investigate a promising and feasible method to determine the phosphatidylcholine (PC) molecular species by RP-HPLC equipped with an evaporative light scattering detector (ELSD). Authentic L-α-PC from soybean was used as the standard for evaluating the individual PC species separation by HPLC. The chromatography was achieved through a C30 column and isocratic mobile phase of acetonitrile/methanol/triethylamine (40:58:2, v/v/v) at a flow rate of 1mL/min, and the detection for ELSD was under the settings of 90°C for drift tube and air flow rate at 1.5L/min. To identify the PC of individual peak on the chromatogram, MALDI-TOF-MS was employed for the detection, and then the results were used to investigate the relationship between retention time and fatty acyl chains of each PC molecule. A linear correlation was observed between retention times and theoretical carbon numbers (TCNs) which can be used to predict the fatty acyl chains of PC molecule. For practical application in the analysis of PC from soybean and sunflower lecithins, the PC was isolated by acetone fractionation and silica gel chromatography. The PC compositions of both lecithins were similar to each other. The dominant PCs were LLPC, OLPC and PLPC.

3. **LC-MS/MS Analysis of Choline/Ethanolamine Plasmalogens via Promotion of Alkali Metal Adduct Formation.** Y. Otoki, K. Nakagawa, S. Kato, and T. Miyazawa, Tohoku University, Japan.

Tandem mass spectrometry (MS/MS) has been used for the analysis of plasmalogen (Pls), a physiologically important class of vinyl ether-linked phospholipid. However, MS/MS generally causes little fragmentation of Pls, especially choline Pls (PC-Pls). Previous MS/MS studies suggest an increased formation of product ions of PC-Pls (and also ethanolamine Pls (PE-Pls)) in the presence of 'alkali metals.' Therefore, use of alkali metals may lead to the development of a method for analysis of both PC- and PE-Pls. In this study, this possibility was evaluated using quadrupole-time-of-flight MS/MS. Results confirmed that alkali metals (e.g., sodium) produced significant fragmentation of PC-Pls and PE-Pls. A number of structure-diagnostic product ions exhibiting high intensities were observed under optimized MS/MS conditions using alkali metals. Moreover, the ability to selectively and sensitively identify PC-Pls and PE-Pls at the molecular species level in biological samples (rat brain and heart) was demonstrated using liquid chromatography coupled with MS/MS. Therefore, the herein developed method appears to be a powerful tool for analyzing Pls and may provide a better understanding of their physiological roles in vivo.