

# 2011 Annual Meeting Abstracts

## Phospholipid

### MONDAY

#### MORNING

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#### PHO 1: Phospholipids in Pharma and Cosmetic Applications

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Chair(s): M. Ahmad, Jina Pharmaceuticals, Inc., USA; and W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands

**Cosmetic Applications of Lecithin-linker Microemulsions.** E.J. Acosta, University of Toronto, Toronto, ON, Canada

Combinations of lecithin with hydrophilic (e.g. C8-PEG) and lipophilic (e.g. sorbitan monooleate) linkers (unbalanced amphiphiles) have been used to produce microemulsions with a wide range of oils. The resulting microemulsions have been found to be biocompatible through a number of tests, including cell viability tests, Human Repeat Insult Patch Test (HRIPT), and Ames test. In this presentation we will discuss the in-vitro and in-vivo performance of an antiwrinkle formula developed using linker-based microemulsions, and the importance of controlling the viscosity of microemulsion-based products for topical applications.

**Synthesis of Well-Defined Amphiphilic Diblock Copolymers Having Biocompatible Phospholipid Polymer Sequences.** Shin-ichi Yusa<sup>1</sup>, Kenichi Fukuda<sup>1</sup>, Kazuhiko Ishihara<sup>2</sup>, Yotaro Morishima<sup>3</sup>, <sup>1</sup>Department of Materials Science and Chemistry, Graduate School of Engineering, University of Hyogo, Hyogo, Japan, <sup>2</sup>Department of Materials Engineering, School of Engineering, The University of Tokyo, Tokyo, Japan, <sup>3</sup>Faculty of Engineering, Fukui University of Technology, Fukui, Japan

2-Methacryloyloxyethyl phosphorylcholine (MPC) is an excellent monomer from a viewpoint of polymerization ability. At first, homopolymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) was synthesized in water by reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. The "living" polymerization was confirmed by the fact that the number-average molecular weight increased linearly with monomer conversion while the molecular weight distribution remained narrow independent of the conversion. The pMPC thus prepared is end-capped with a dithioester moiety. Using the dithioester-capped pMPC as a macro-chain transfer agent, AB diblock copolymers of MPC and n-butyl methacrylate (BMA) were synthesized. Associative properties of the amphiphilic block copolymer (pMPCm-BMA) with varying pBMA block lengths were investigated using NMR, fluorescence probe, and light scattering techniques. The formation of polymer micelles comprising a core with pBMA blocks and shell with hydrophilic pMPC blocks was suggested by light scattering data. Since excellent biocompatible pMPC sequences form an outer shell of the micelle, pMPCm-BMA may find an application as a promising reagent to make a good formulation with a hydrophobic drug.

**Rheological Characterization of Oil-Wax Gels for Cosmetics.** Y. Miyazaki<sup>1</sup>, K. Yoshida<sup>1</sup>, A. Marangoni<sup>2</sup>, <sup>1</sup>Kao Corporation, Sumida-ku, Tokyo, Japan, <sup>2</sup>Dept. of Food Science, University of Guelph, Guelph, ON, Canada

The relationship between the rheological properties and micro- and nano-structure of two wax-oil gels was studied. These gels are commonly used in cosmetics especially in lipsticks since the waxes keep the cylindrical shape while the oils provide luster and moisture retention. In contrast, the food industry uses waxes as coating agents in fruits and cheese to provide a glossy look or protection from physical damage or water loss. The two systems under study were Paraffin wax-oil (SW gel) and Polyethylene wax-oil (PW gel). The two waxes differ in chain length and the spatial distribution of alkane molecules. Rheological studies showed that the SW gel was stiff and fragile, whereas PW gel was soft and malleable. To explain the difference, the micro-structure was studied with cryo Scanning Electron Microscope (cryo SEM), and the nano-structure was studied using Powder X-ray Diffraction (XRD). The relationship between the elastic modulus and the mass density was modeled using cellular solid models. The characteristic exponent suggested that the SW was an open cell foam, while the PW was identified as an open cell foam with cell wall defects. These predictions agreed with SEM observations that SW crystals produced thicker walls and more apparent junctions between platelets than PW. XRD results also demonstrated wax molecular lamellae only in SW, which correlated with effects at larger length scales.

**Phospholipid Nanopharmaceuticals in Advanced Drug Delivery.** H. Mansour, University of Kentucky College of Pharmacy, Lexington, KY, USA

This talk will present the fundamentals and applications of phospholipids in pharmaceutical nanomedicine delivery. Advanced particle engineering design technologies used to create pharmaceutical phospholipid powders will be discussed in the context of solid-state phospholipid properties, particle properties, biophysical properties and pharmaceutical performance. Phospholipid self-assembly behavior and novel nanomedicine applications will also be discussed.

## AFTERNOON

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### PHO 2: Structured Lipids and Phospholipids

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Chair(s): X. Xu, University of Aarhus, Denmark; and S. Ali, Jina Pharmaceuticals, Inc., USA

**Influence of the Reaction Conditions on Enzymatic Synthesis of Glycerolipids.** Patrick Adlercreutz, Lund University, Lund, Sweden

Lipases and phospholipases are excellent tools in lipid synthesis. The regioselectivity of the enzymes make them especially useful in preparation of structured glycerolipids. New examples of such lipids with interesting physico-chemical properties and/or potential health promoting effects will be presented. The enzymatic reactions involve partially deacylated lipids as intermediates and therefore acyl migration is a possible side reaction. This sometimes causes

problems, but it can also be used for the deliberate preparation of new products. The influence of reaction parameters such as water activity, temperature and the presence of support materials and other additives on the enzymatic reactions and potential side reactions will be discussed.

**Chemo-Enzymatic Synthesis of Polymerizable Structured Lipids.** V. Mannam, D.G. Hayes, University of Tennessee, Knoxville, TN, USA

We have been preparing star polymers consisting of oligo(hydroxy fatty acyl) chains conjugated to polyols enriched in primary hydroxyls such as pentaerythritol for as a possible vehicle for delivery of lipophilic drugs. A problem with the "first generation" star polymers is the low number of oligo(hydroxyacyl) chains per molecule, limited by the absence of polyols containing > 4 primary OH groups. As an alternative approach, we have formed via a chemo-enzymatic process structured triacylglycerols (TAG) that contain 2 oligo(ricinoleic acyl) groups and 1 11-undecenoic acyl group. The structured TAG can be subsequently tethered together through the undecenoic acyl groups via free radical polymerization. Two different chemo-enzymatic approaches have been investigated for structured TAG preparation: the immobilized *Rhizomucor miehei* lipase (RML)-catalyzed interesterification of castor oil and vinyl undecenoin, followed by oligomerization of the TAG ricinoleic acyl groups through adding ricinoleic acid and employing immobilized *Candida antarctica* lipase (CAL). The second approach is the RCAL-catalyzed synthesis of triundecenoin (via alcoholysis of glycerol by vinyl undecenoin), followed by RML-catalyzed interesterification of triundecenoin and ricinoleic acid, followed by CAL-catalyzed oligomerization between ricinoleic acid and the TAG's ricinoleic acyl groups.

**Enzymatic Production of Commercial Structured Lipids.** T.K. Yang, Wilmar Global R & D Center, NO 118 Gaodong Road, Pudong, Shanghai 200137, China

This review deals with the commercial process of structured triacylglycerols (STGs) including MLM-type STGs, MLM-type STG containing EPA, MLM-type STG containing DHA, LML-type STGs and human milk fat substitutes. MLM containing EPA (or DHA) can be synthesized from glycerol, medium chain fatty acids or its ethyl ester and EPA (or DHA) or its ethyl ester, catalyzed by 1,3-specific lipase. The enzymatic synthesis of pure MLM-type STGs mainly includes: preparation of simple TG, and then replacement of FA residues at the 1,3-positions, leaving the one at the 2-position unchanged. The approach to mimic triacylglycerol structure found in human breast milk should be carried out using enzymatic interesterification with high specificity and mild reaction conditions.

**Biocatalytic Route to Surface Active Lipid.** Ling-Zhi Cheong, Xuebing Xu, Department of Molecular Biology, Aarhus University, Aarhus, Denmark

Lipid can be structurally modified in order to attain improved functional properties. This work look into the possibilities of developing surface active lipids with improved functional properties through biocatalytic route. Biocatalytic route to surface active lipid are usually complex involving a biphasic system as the substrates used may differs in polarity. In some instances, chemical synthetic route are used. Attempts are made to study the biocatalytic route to surface active lipid including various factors affecting synthesis of such compounds. The surface active

lipids produced may have distinct self assembling property and find useful application in surfactant industry.

**Production of Structured Lipids from Different Plant Oils Containing Conjugated Linolenic Acid Originated Bitter Gourd and Pomegranate Seed Oils.** H.A. Aksoy, G. Ustun, M. Tuter, Istanbul Technical University Chemical Engineering Department, Istanbul, Turkey

Enzymatic production of structured lipids (SLs) containing conjugated linoleic acid (CLA) was extensively studied. Although conjugated trienes are stronger anticarcinogens than conjugated dienes, in literature no record of study on conjugated linolenic acid (CLNA) incorporation into oils has been encountered. Therefore, we have attempted to investigate CLNA incorporation into different plant (corn, canola, hazelnut and olive) oils to enhance their health benefits with CLNA of bitter gourd (*Momordica charantia* L.) and pomegranate (*Punica granatum* L.) seed oils by acidolysis using Lipozyme TL IM (*Thermomyces lanuginosa*). The major CLNA isomer of bitter gourd seed oil is alfa-eleostearic acid while that of pomegranate seed oil is punicic acid. Origin of CLNA did not effect CLNA incorporation into corn and canola oils and the highest CLNA incorporation (31-33%) was resulted at 50 C in hexane using 10% enzyme at 1:5 substrate molar ratio and 3 h. At these reaction conditions, CLNA originated from pomegranate oil was incorporated into olive and hazelnut oils resulting SLs containing total CLNA of 45 and 49.2 %, respectively. These SLs could be used as a source of dietary CLNA in the formulation of food products.

**Nutraceutical Effects of Structured Lipids.** Mahua Ghosh, Avery Sengupta, University of Calcutta, Kolkata, West Bengal, India

Structured lipids are defined as triacylglycerols which are modified chemically or bio-chemically to alter the composition/or position of the fatty acids in the glycerol backbone. Sometimes blending of two or more lipid components also produces structured lipids. Their importance lies in providing improvements in health, in non-storage of excess energy in adipose tissues, in plasma lipid profiles and in blood parameters etc. Hypolipidemic and antioxidative properties of PUFA and medium chain fatty acid (MCFA) are already established. The present study will demonstrate the haematological, histological and rheological property of erythrocyte membrane of PUFA rich and MCFA rich mustard oil in hypercholesteromic rat model. Significant abnormality in haematological examinations such as platelet count, haemoglobin level, haematocrit value, platelet aggregation were observed in hypercholesterolaemia which were partially normalized by the administration of experimental oils. Organopathological examination showed that there was deposition of cholesterol in the liver in hypercholesterolaemic condition, which was also reduced by treatment with the two experimental oils. Osmotic fragility data suggested that membrane fragility increased with hypercholesterolemia which could be reversed with the addition of experimental oils in the diet.

**TUESDAY**

**AFTERNOON**

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**PHO 3 / H&N 3.2: Applications of Phospholipids with n-3 Fatty Acids**

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Chair(s): T. Wang, Iowa State University, USA; G. Wang, Cargill, USA; and J. Whittinghill, Solae, LLC, USA

**Nutritional Properties of Phospholipids with n-3 Fatty Acids.** T. Wang, Iowa State University, USA

Phospholipids (PLs) are health-enhancing nutrients. PC and PE maintain and promote the function of central nerve system. In recent years, PLs have also been shown to be good carriers and delivery means for enriching omega-3 fatty acids (FAs) in blood lipids. Evidences are provided in this review from several original research papers. PLs with omega or n-3 FAs are also shown to have anti-obesity and anti-cancer effects.

**Lipase Catalyzed Production of Lysophospholipids Rich in DHA Originating from a Marine Microalga.** L. Poisson, F. Ergon, G. Pencreac'h, IUT de Laval - MMS (EA 2160), Laval, France

Nutritional interest of  $\omega$ 3 polyunsaturated fatty acids (PUFAs) is no longer in question among scientists however the form under which these  $\omega$ 3 PUFAs should be delivered to be as efficient as possible needs further investigations. Nevertheless it seems that phospholipids as well as lysophospholipids are possible good carriers for  $\omega$ 3 PUFAs and it seems that docosahexaenoic acid (DHA,  $\omega$ 3 C22:6) is less subject to peroxidation when linked to phospholipids than to triglycerides or ethyl esters. The use of lipase for the production of lysophospholipids rich in DHA originating from the marine microalga *Isochrysis galbana* has been studied. Lipid analysis was first carried out to define the content of DHA in the phospholipid fraction and the position of DHA on the phospholipid backbone. A screening of 12 lipase preparations was then implemented with a view to highlight enzymes leading to a discrimination between DHA and others fatty acids within phospholipid deacylation. A lysophospholipid fraction containing 79% of DHA was obtained with a recovery yield of 85%. The phenomena of non enzymatic acyl migration during phospholipid deacylation was also investigated with a view to lower the content of non desirable final products.

**Delivery of Bioactive Compounds through Milk Phospholipids Nanoliposomes.** Bita Farhang, Yukio Kakuda, Milena Corredig, University of Guelph, Guelph, ON, Canada

Liposome technology is an effective technology for encapsulation of bioactive compounds. This research is focused on liposomes prepared from milk phospholipids which can be considered of great nutritional value per se, because of high content in sphingomyelin. Nanoliposomes were prepared using microfluidization, and  $\beta$ -Carotene and Vitamin C were incorporated as model hydrophobic and hydrophilic compounds. The results show high incorporation efficiencies for  $\beta$ -Carotene and high stability of vitamin C when entrapped in the liposomes. The incorporation efficiency of 26.5% for vitamin C was obtained with 10% milk phospholipids. About 70 % of encapsulated vitamin C remained after storage at 4°C and pH 7 for 7 weeks, without significant increases in peroxide value and liposome size. Incorporation of  $\beta$ -Carotene yielded larger nanoliposomes and by simultaneous incorporation of  $\beta$ -Carotene and Vitamin C, the incorporation efficiency of 92 % was obtained for  $\beta$ -Carotene without any significant change in

vitamin C encapsulation. These results would suggest that  $\beta$ -Carotene enhanced the lamellar size of the liposomes, without increasing the lumen volume. As fractions of milk phospholipids derived from the milk fat globule membrane become commercially available, there will be an increased demand for these ingredients for the preparation of liposome material as a delivery system.

**The Antioxidant Effect of Phosphatidylserine in Refined Fish Oil.** A.J. Reid<sup>1</sup>, S.M. Budge<sup>1</sup>, M. St Onge<sup>2</sup>, <sup>1</sup>Dalhousie University, Halifax, NS, Canada, <sup>2</sup>Ascenta Health Ltd., Dartmouth, NS, Canada

Increasing the oxidative stability of fish oils is economically and nutritionally important with the increase in consumer knowledge of the value of long chain omega-3 fatty acids. Phosphatidylserine has been investigated for its antioxidant/synergistic effect on oil stability. Literature indicates that there is a synergistic relationship between tocopherols and phospholipids, including phosphatidylserine. The individual and combined effect of phosphatidylserine and tocopherols on the oxidation stability of refined triacylglycerol (TAG) fish oil was examined. Oil was incubated in the dark at 40°C and oxidation was monitored by following changes in peroxide and p-anisidine values. Tocopherol levels were also measured to determine their decomposition over the oxidation period.

**Extraction of Phospholipids from Egg Yolk: Effect of Solvent and Drying Treatment.** H. Wang<sup>1</sup>, L. Yao<sup>2</sup>, T. Wang<sup>2</sup>, <sup>1</sup>Center for Crops Utilization Research, Iowa State University, Ames, IA 50010, USA, <sup>2</sup>Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50010, USA

Two solvents, hexane and ethanol, were used to extract lipids from two types of pre-dried egg products, drum-dried egg yolk and pelletized spray-dried whole egg powder. The phospholipids were precipitated using acetone at two temperatures. The purity of the phospholipids fraction was evaluated by NMR. The effects of different pre-drying treatment, solvent types, and acetone precipitation temperature on the yield and purity of egg phospholipids will be presented and discussed. This research was designed to develop a commercially feasible method to extract phospholipids from chicken eggs.

**Role of Phospholipids Reverse Micelles on Lipid Oxidation: Impact of Minor Components on Physicochemical Properties of Stripped Soybean Oil.** B.C. Chen, D.J. McClements, E.A. Decker, Department of Food Science, University of Massachusetts, Amherst, MA, USA

Phospholipids self-assemble in bulk oils to form reverse micelles that can alter the microenvironment where chemical degradation reactions occur, such as lipid oxidation. In this study, we examined the influence of phospholipid reverse micelles (1) on the activity of non-polar ( $\alpha$ -tocopherol) and polar (Trolox) antioxidants; and (2) on the interaction of minor components, i.e., diacylglycerols, monoacylglycerols and free fatty acids in stripped soybean oil (SSO). Reverse micelles were formed by adding 1000 microM 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to SSO. The addition of DOPC reverse micelles had a prooxidant effect, shortening the lag phase of SSO at 40, 55 °C. Small angle X-ray scattering (SAXS), fluorescence life time decay, interfacial tension were used to characterize the physical structure

changes when SSO incorporated with reverse micelle encountering with minor component individual or combinations. Lipid hydroperoxides and volatile hexanal were measured under different conditions to understand the oxidation kinetics of SSO. These results will improve our understanding and control of lipid oxidation in bulk oils.

**Efficient Enzymatic Synthesis of Phenolic Ester by Increasing Solubility of Phenolic Acids in Ionic Liquids.** Zhiyong Yang, Zheng Guo, Xuebing Xu, Department of Molecular Biology, Aarhus University, Aarhus, Denmark

Compounds from phenolic acid family are well known natural antioxidants, but the application of phenolic acids as antioxidants in industry is limited due to the relatively low solubility in oil-based media. The properties of phenolic acids can be modified through enzymatic lipophilization and modified phenolic acids will have amphiphilic property, therefore they can be localized at oil-water or water-oil phase where oxidation is considered to occur frequently. It had been reported that immobilized *Candida Antarctica* lipase B was the most effective biocatalyst for the various esterification reactions, and it had been widely used for esterification of various phenolic acids with fatty alcohol or triglycerides. However, the conversion of phenolic acids is low due to low solubility in hydrophobic solvents and hindrance effect of unsaturated side chain towards the enzyme. Our studies show these barriers can be overcome by increase the solubility of phenolic acids. Ionic liquid Methyltrioctylammonium Trifluoroacetate can dissolve different phenolic acids in very high concentration. This ionic liquid was therefore applied for esterification of phenolic acids with fatty alcohol or triglyceride.

**Lipid Vesicles with High Entrapment Efficiency Prepared by Using Emulsions.** Sosaku Ichikawa, University of Tsukuba, Tsukuba, Ibaraki, Japan

Lipid vesicles (also called liposomes) can be utilized as carrier of functional materials. However, the preparation of size-controlled vesicles containing hydrophilic materials with high entrapment efficiency is rather difficult. In this presentation, two types of methods for preparing lipid vesicles using emulsions are presented. One is "Vesicle formation from W/O/W multiple emulsions" and other is "Vesicle formation by lipid-coated ice droplet hydration method". In the former method, W1/O/W2 emulsion was prepared by microchannels emulsification that enables to reduce the leakage of to-be-entrapped materials from W1 phase to W2 phase. The organic solvent in the oil phase (O) was evaporated to form vesicles. With this procedure, vesicles with high entrapment efficiency, namely over 90% for 200 nm-sized, could be formed. The size reflects the water droplet (W1) size. In the latter method, giant vesicles (GVs) could be formed by the hydration of lipid-coated ice droplet containing to-be-entrapped materials. Carboxylesterase as a model enzyme was entrapped in GVs, and the enzymatic reaction inside GVs was conducted. These methods enable the preparation of rather large amount of vesicles with high entrapment efficiency and controlled-size, potentially useful in the research and practical fields of food-, cosmetic- and pharmaceutical- industries.

**PHO 3.1: Symposium on NMR in Lipids**

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Chair(s): B. Diehl, Spectral Service AG, Germany; and G. Knothe, USDA, ARS, NCAUR, USA

## **NMR Principles in Lipid Analysis.** B.W.K.-H. Diehl, Spectral Service AG, Germany

Lipids are a prime example for classical multi-component analysis. The test item "lipid" is of central importance for the analysis of food and feed but also for drugs and technical chemicals. The combination of neutral and polar lipids, sterols and other biomolecules from animal and vegetable sources demands a comprehensive regulation opus of official methods, e.g. by the DGF (Deutsche Gesellschaft für Fettforschung) or the AOCS (American Oil Chemists' Society). This collection of official methods has the advantage of a worldwide comparable analysis of oils and lipids. On the other hand the commitment to this selection of methods can be a disadvantage. Technical and scientific scopes in lipid analysis had changed during the time and new and more effective methods became available. They also have to be accepted and implemented as official methods in the future. The presentation shows the principles of NMR spectroscopy in lipid analysis using 600 MHz cQNP probe for the nuclei  $^1\text{H}$ -  $^{13}\text{C}$ - and  $^{31}\text{P}$  and provides the basic background for the following presentations of the NMR symposium.

**$^1\text{H}$ -NMR Method to Determine the Hydroperoxide Amount of Edible Oils.** C. Skiera<sup>1,2</sup>, P. Steliopoulos<sup>2</sup>, T. Kuballa<sup>2</sup>, B.W.K-H. Diehl<sup>3</sup>, U. Holzgrabe<sup>1</sup>, <sup>1</sup>Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Germany, <sup>2</sup>Chemisches und Veterinäruntersuchungsamt Karlsruhe, Karlsruhe, Germany, <sup>3</sup>Spectral Service AG, Cologne, Germany

Lipid oxidation is the most important cause of oil and fat deterioration, which reduces shelf life and nutritional value. During the autoxidation process of lipids, hydroperoxides are formed as primary products that are easily decomposed to secondary products such as aldehydes, ketones, alcohols, and acids. The peroxide value (PV) according to Wheeler is routinely used as an indicator of the early stages of oxidation in edible fats and oils. This parameter is expressed in milliequivalents peroxide per kg and covers all compounds that oxidize potassium iodide under defined conditions. As an alternative to the PV, some authors propose the  $^1\text{H}$ -NMR-determined ratio between the olefinic and aliphatic protons and the ratio between the aliphatic and diallylmethylene protons in fatty acids. A good correlation between these ratios and the classical PV has been found. Yet, this method is not sufficiently sensitive. In this study, we present a new, efficient  $^1\text{H}$ -NMR assay to determine the hydroperoxide amount in edible oils. We investigated the effect of solvent and of impurities on peak width and chemical shift of the hydroperoxide proton signal (OOH). Furthermore, several oils and oil mixtures were examined by both the classical Wheeler approach and the  $^1\text{H}$ -NMR method in order to compare the relative sensitivity of the methods.

**NMR Analysis of Biodiesel.** Gerhard Knothe, USDA, ARS, NCAUR, Peoria, IL 61604, USA

Biodiesel is usually analyzed by the various methods called for in standards such as ASTM D6751 and EN 14214. Nuclear magnetic resonance (NMR) is not one of these methods. However, NMR, with  $^1\text{H}$ -NMR commonly applied, can be useful in a variety of applications related to biodiesel. These include monitoring of the transesterification reaction that produces biodiesel and related quality issues, determining the fatty acid profile to a large extent, assessing the oxidation status and verifying levels of blending with petrodiesel. These issues, including a comparison to those prescribed in standards, will be addressed.

## **Analysis of Emulsifiers E484 and E433 in Animal Feed, a NMR and HPLC/MS Study.**

Bernd W.K-H. Diehl, Gabriele Randel, Spectral Service AG, Cologne, Germany

Castor Oil Polyethylenglycole (E484) and Polyoxyethylensorbitanmonooleat (E433) are emulsifiers used in animal feed. The analysis is complicated because each type of emulsifier consists of a complex mixture of natural materials, which underwent oligomer and polymer reactions. The fatty acid composition, the degree of esterification and the mean chain length vary depending on the raw material and the conditions for the polymerisation processes. The mean ethylene glycol units per molecule castor oil vary from 10 to 200. In addition modified e.g. hydrogenated castor oil may be used as basic molecule. The ethoxylation process influences the fatty acid composition due to isomerisation of the double bonds from natural cis-homoconjugated to cis/trans-conjugated species. Other chemical reactions like elimination and hydrolysis result in the fact, that emulsifiers summarised as E484 or E433 in fact are complex mixtures of hundreds of single molecules. However, for regulatory purposes ? to avoid the use of other illegal emulsifiers - two independent orthogonal analytical methods had been developed to analyse and distinguish both types of emulsifiers. The present study presents the results of NMR spectroscopy (<sup>1</sup>H NMR and <sup>13</sup>C NMR) in combination with HPLC/MS chromatography to solve the problem satisfying.

### **Panel Discussion.**

## **WEDNESDAY**

### **MORNING**

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#### **PHO 4: Phospholipids in Food and Nutraceutical Applications**

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

**A Review of The Multifunctional Properties of Lecithin in Food Systems.** L. Colbert, Archer Daniels Midland, Decatur, IL, USA

Since its first isolation by Theodore Nicolas Gobley in 1846, lecithin has gained an increasing role as an emulsifier in food processing systems. This treatise is a review of the chemical and physical properties which enable lecithin to effectively perform as an emulsifier, instantizer, and release agent in food systems. A knowledge of the effects of HLB on the nature of the emulsification properties is key to understanding the usage of different lecithin types. A knowledge of the manner of lecithin inclusion, and partnerships with other emulsifiers unravels some of the mystery of such complex systems as bakery, and beverages. Enhancement of the above properties through modification, and fractionation are discussed.

**Emulsifying Properties of Different Modified Sunflower Lecithins.** D.M. Cabezas<sup>1</sup>, R.

Madoery<sup>2</sup>, B.W.K. Diehl<sup>3</sup>, M.C. Tomás<sup>1</sup>, <sup>1</sup>Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas (UNLP-CONICET), La Plata, Pcia. de Buenos Aires, Argentina, <sup>2</sup>Cátedra de Química Orgánica, FCA UNC, Córdoba, Argentina, <sup>3</sup>Spectral Service, Cologne, Germany

Lecithin is a multifunctional ingredient used in a wide range of industrial applications. Most commonly processes for lecithin modification are deoiling; fractionation with alcohol or enzymatic hydrolysis. The aim of this work was to evaluate the emulsifying properties of different modified sunflower lecithins (MSL) in O/W emulsions. Five MSL obtained by deoiling, fractionation with absolute ethanol (PC and PI enriched fractions), and enzymatic hydrolysis with phospholipases A<sub>2</sub> from pancreatic porcine and microbial sources (hydrolyzed lecithins) were assessed. Phospholipid composition of samples was determined by <sup>31</sup>P NMR. Modified lecithins were applied as emulsifying agent in O/W emulsions (30:70 wt/wt), ranging 0.1-2.0% (wt/wt). Emulsion stability was evaluated through the evolution of backscattering profiles, particle size distribution, and mean diameters (D [4,3], D [3,2]). PC enriched fraction and both hydrolyzed lecithins presented the best emulsifying properties against the main destabilization processes (creaming and coalescence) of the emulsions studied. This fact could be related to the higher concentration of hydrophilic phospholipids (PC, lysophospholipids) presented by MSL respect to native sunflower lecithin, which enhance their hydrophilic-lipophilic balance (HLB). MSL represent a good alternative for the production of new bioactive agents.

**Evaluation of Deoiled Lecithin in the Production of Flour Tortillas.** Bruce Sebree, ADM Research, Decatur, IL, USA

Flour tortillas were produced according to commercial formulations and procedures at AIB. Four treatments (0.5, 1.0, 1.5, and 2.0%) utilizing deoiled lecithin were produced, with a negative control. All doughs were produced under controlled conditions in duplicate. Doughs were subjectively evaluated for handling characteristics during mixing and at make-up. Tortillas were subjectively evaluated for external, internal and eating quality characteristics one day after baking. Tortilla diameter was measured one day after baking. Product quality was evaluated on days 1, 4, 7 and 10 after baking. Flour tortillas were evaluated for texture differences using the TA-XT2 Texture Analyzer to measure grams of force to break and stretch tortillas. Dough handling and external characteristics of treatments and control were equal. Control was noted as "tough" regarding mouth-feel versus "moist" for 0.5, 1.0 & 1.5% and "tender" for 2.0% treatments. For stickiness, the 2.0% treatment was noted "less sticky" and balance were "sticky". Elasticity over 10 days - the 0.5% treatment required the most force to break and had 1st or 2nd highest stretching. Overall, the 0.5% treatment produces a tortilla with good elasticity and moistness. Depending upon characteristics that a producer is looking to achieve, other dosings may be appropriate.

**Phospholipids in Functional Beverages.** Dianne Bukowski, American Lecithin Company Inc., Oxford, CT, USA

Phospholipids are natural substances that are essential to life. They are important structural components of cellular membranes. In addition to their biological and physiological properties, phospholipids provide functionality in numerous ways to Functional Beverages. In beverages,

Phospholipids function as natural emulsifiers, wetting and instantizing agents. In addition to their stabilizing properties, phospholipids and phospholipid derived ingredients play important nutritional roles. The uses and functionality of Phosphatidylcholine (PC), Phosphatidylserine (PS) and Glycerophosphocholine (GPC) as nutritional ingredients will be discussed.

Phospholipid-based delivery systems to introduce lipophilic active ingredients such as fat-soluble vitamins, specific phytochemicals, and omega-3 fatty acids into beverage will also be discussed.

**Lecithin Quality Sourcing Issues from a European View.** W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands

The best quality of liquid lecithin has a light red colored glossy and transparent appearance, obtained by careful processing of intensively filtered extracted soybean oil. This standard is offered by leading N-American producers and in the last decades by some European mills. The intensive filtration with filter aids is expressed by Hexane / Toluene Insoluble matter well below the legal standard of maximum 0.3%. This characteristic enables also the further processing to deoiled lecithin powder or stable liquid compositions. Of course the Phospholipid composition is also important for achieving the best emulsification performance. Nowadays European food processors require lecithins from IP non-GMO sourced oil seeds, by which the search for soy lecithin in other continents and from sunflower and canola seeds intensified during the last decade. Those lecithin qualities need also intensive filtration, degumming and drying equipment for processing qualities, complying with highest food ingredient standards. Equipment, processing and handling details will be discussed.

## AFTERNOON

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### PHO 5: General Phospholipids

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Chair(s): L. Colbert, Archer Daniels Midland Co., USA; and TBD

**Greener Agricultural Adjuvants Using Phospholipids- A Growing Market Trend.** Shireen Baseeth, Bruce Seabee, Archer Daniels Midland, Decatur, IL, USA

Agrochemical formulations and delivery systems are evolving to meet the new standards required by their users, producers, and regulatory agencies. There has been a rise in innovative and nontraditional approaches to deliver pesticide products due to these and other factors. The current market situation could be called "the era of invention in agrochemicals" where continually improved formulations are essential for success. More of this focus is now towards developing renewable/sustainable products that are benign to the human health and environment. Since lecithin is a food grade emulsifier it offers major technical, process and product safety advantages for good agricultural practice. With the growing interests in renewable feed stocks, lecithin meets all the needs for new product development in more competent areas of application. In this presentation we report the performance properties of a lecithin based agricultural spray adjuvant. The product's superior ability in all adjuvant functions uniquely positions it as an ideal tank mix partner. Superior efficacies are achieved by providing measurable results in several areas like droplet size and coverage, deposition and retention, acidification and penetration,

wetting/spreading and drift control.

**Enrichment of Phospholipids from Biological Matrices with Zirconia-modified Silica Sorbents Followed by LC-MS/MS Analysis.** X. Lu, Supelco/Sigma Aldrich, USA

**Optimisation of Enzymatic Degumming by Applying a New Understanding of Reaction Kinetics.** W.D. Cowan<sup>1</sup>, H.S. Yee<sup>2</sup>, H.C. Holm<sup>3</sup>, <sup>1</sup>Novozymes UK, Chesham, Bucks, UK, <sup>2</sup>Novozymes Malaysia, Kuala Lumpur, Malaysia, <sup>3</sup>Novozymes A/S, Bagsvaerd, Denmark

When originally developed Enzymatic Degumming required long reaction times and high investments in equipment. Recent studies have demonstrated that reaction times can be reduced substantially. However the conversion of phospholipids to their lyso form and the optimum degree of modification was not well understood. This paper reports new findings on the reactions occurring and the limited generation of free fatty acids resulting from the modified reaction time. In addition the expansion of this technology to the preparation of oils for bio-diesel is introduced as a significant advance in improving the efficiency of the conversion of oils to FAME.

**Pressure Perturbation and Differential Scanning Calorimetric Studies on Phospholipid-Peptides Mixtures.** L.N. Okoro, American University of Nigeria, Yola, Adamawa, Nigeria

The effect of gramicidin D (gD) and melittin incorporated into the phospholipid bilayer DPPC at mole fractions up to 10 mol% and 3.75 mol% have been investigated. The study reveals a considerable influence of gD and melittin on the phase transition profile and volumetric properties of the DPPC bilayer. The thermal coefficient of expansion, and the volume changes of DPPC ? gD and DPPC ? melittin bilayer membranes in their different transition phases were determined by using pressure perturbation calorimetry (PPC), a relatively new and efficient technique, and differential scanning calorimetry (DSC). The experiments were carried out in the temperature range from 10 to 85°C. Two endotherms are present in the DSC thermogram at 5 mol% gD concentration, and the effect of addition of up to 10 mol% of gD is a symmetric broadening of the heat capacity. Incorporating melittin up to 1.25 mol% into the lipid bilayer abolished the pretransition. Remarkably, there is no shift in the transition temperature up to 2.5 mol% melittin, with more than one shoulder observed at the low-temperature side of the DSC peak for 3.75 mol%, and a shift in melting temperature,  $T_m$  to 32°C. This could be linked to the lytic property of melittin at these high peptide concentrations.

## Phospholipids Posters

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

**Storage Stability of Marine Phospholipids Emulsions.**

F.S. Henna Lu, N.S. Nielsen, C.P. Baron, C. Jacobsen, Technical University of Denmark, Kgs. Lyngby, Denmark

Marine phospholipids (MPL) are believed to provide more advantages than fish oil from the same source. They are considered to have a better bioavailability, a better resistance towards oxidation and a higher content of polyunsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) than oily triglycerides (fish oil). Therefore, the objective of this study is to explore the feasibility of using marine phospholipids emulsions as a delivery system through investigation of the physical, oxidative and hydrolytic stability of MPL emulsions with or without addition of fish oil. The effect of initial Peroxide Value, total lipids, phospholipids and antioxidants content on stability of MPL emulsions were studied. The physical stability was investigated through measurement of particle size distribution and creaming stability, which involve measurement of changes (%) in emulsion volume. In addition, preliminary investigation of the oxidative and hydrolytic stability was carried out through determination of Peroxide Value and Free Fatty Acids Value after 32 days storage at room temperature and 2°C, respectively. Oxidative stability of MPL emulsions were also investigated through measurement of secondary volatile compounds by Solid Phase Microextraction at several time intervals at 2°C storage. Preliminary results showed that MPL emulsion has a good oxidative stability.