PHO 1: Analytics of Phospholipids

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

Monitoring of the Phospholipid Oxidation in Liposomes by NMR Spectroscopy. B.W.K. Diehl, Spectral Service AG, Cologne, Germany.

The detection and quantification of phospholipid oxidation is not successful using the classical titration methods for lipids. In liposomal formulations these analysis are more complex; therefore there is a need of a new method. Since ^{1}H-NMR is equivalent for the detection of peroxide values in edible oils NMR spectroscopy is used for monitoring the oxidative stability of liposomal formulations with phosphatidylcholine. The combination of ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR documents the primary oxidation step to peroxides and the secondary for aldehydes.

^{31}P-NMR Round Robin Test on Krill Oil. B.W.K. Diehl, Spectral Service AG, Cologne, Germany.

Ten different NMR laboratories participated in the first ^{31}P-NMR round robin test on quantitative NMR of krill oil phospholipids according USP using NMR instruments with field strength between 4010 and 600 MHz. Krill oil is a complex mixture of approx. 50% phospholipids. PC is the major compound, 10% of the PC is an ether derivative. All laboratories demonstrate its ability to perform the tests in a proper way. The mean standard deviation for the PC signal was 1.2% and of the total phospholipids of 1.0 %. The test was done under the supervision of the I.L.P.S.

Novel Orthogonal Technologies in Support of Mass Spectrometry-based Lipid Analysis. G. Astarita, A. Doneanu, G. Isaac, J. Johnson, J. Murphy, and J. Langridge, Waters, Milford, MA, USA.

Lipidomics is the comprehensive analysis of lipids in food, algae, biological tissues and microorganisms with applications in pharmaceutical, chemical, clinical, nutrition and food sciences. Mass Spectrometry (MS) is the technique of choice for lipidomic analysis. Novel orthogonal chromatographic tools are supporting MS in the characterization of lipid species. In particular, ultra performance convergence chromatography (UPC^2), which uses liquid CO_2 as mobile phase, and represents an evolution of supercritical fluid chromatography, in terms of speed and reproducibility of analysis. UPC^2 is enabling new ways of separating small lipids and phospholipids. Another novel chromatographic tool is an integrated microfluidic device for lipidomics applications, which offers the advantages of increased sensitivity, decreased solvent consumption, and lower injection volume over traditional UPLC. Both UPC^2 and microfluidics technologies operate at an environmentally friendly, cost-saving, scale with respect to solvent consumption and waste disposal. Having such advantages, while maintaining UPLC-like performance in terms of chromatographic resolution, robustness and reproducibility of analysis, could be a real game-changer for chromatography and for lipid analysis. Additional orthogonal technologies such ion mobility-MS will also be reviewed for lipid analysis.

Phospholipid Analysis in Food Standards – HPLC or NMR? K.B. Laurvick, US Pharmacopeia, Rockville, MD, USA.

Public standards for “lecithin” ingredients intended for use in foods and excipients are available from multiple sources, including *USP-NF*, JECFA, and the *Food Chemicals Codex*. These standards are intended to describe the minimum purity and quality of lecithin ingredients, which are used in the manufacture of many types of foods. Because “lecithin” is a nonspecific term, public standards have historically been able to describe physical attributes of the ingredients, lacking analytical techniques that are both specific and quantitative for the unique phospholipids present in different food ingredients. Recently standard methods have been developed by industry associations that allow quantitation of phospholipids by HPLC and NMR analyses. In an effort to provide users with methods for determining a more specific composition of lecithin products, the U. S. Pharmacopeial Convention (USP) has worked to incorporate these analytical techniques into its compendia the *Food Chemicals Codex* and *USP-NF*. These efforts have resulted in a modernized monograph for Lecithin (HPLC analysis) in the *USP-NF* compendium and a monograph for Krill Oil (NMR analysis) in the *Food Chemicals*. The complex chemistry of phospholipid analysis will likely lead to

more specific public standards for these and other phospholipid-based food and drug ingredients – work that can be supported by public standards organizations.

PHO 2: Phospholipid Novelties

Chair: M. Tomas, CIDCA-UNL, Argentina

Novel and Efficient Solid to Solid Transphosphatidylation of Two Phenylalkanols in a Biphasic GRAS Medium and a Comparative Study of the Antioxidant Activity of Phosphatidyl Derivatives of Hydroxytyrosol. V. Casado[1], D. Martín[1], L. Vázquez[1], A. Garcia-Serrano[1], G. Reglero[1,2], and C.F. Torres[1], [1]Departamento de produccion y caracterizacion de nuevos alimentos, Instituto de Investigacion en Ciencias de la Alimentacion (CSIC-UAM), Madrid, Spain, [2]IMDEA-Food Institute, Madrid, Spain.

A solid to solid reaction system for transphosphatidylation of phosphatidylcholine with two different phenylalkanols, namely tyrosol and hydroxytyrosol (HT) has been developed. The enzymatic reactions were carried out in the presence of a food grade phospholipase D at very mild reaction conditions (40 ºC). All enzymatic reactions were carried out in a biphasic GRAS medium comprised of sodium acetate buffer and ethyl butyrate. High volumetric productivity (up to 130 g of phosphatidylcholine per liter of reaction mixture) and equimolar ratio of substrates have been utilized. At these reaction conditions, up to 150 mmol/L of both, phosphatidyltyrosol and phosphatidylhydroxytyrosol (PHT) were obtained.

In addition, a comparative study of the antioxidant activity of phosphatidyl derivatives of HT (PHT) and HT added at increasing concentrations to diverse edible oils (lard oil –LO-, refined olive oil –OO-, and diacylglycerol-rich oil –DO-) was performed. PHT and HT showed a protective effect as the added concentration of antioxidants increased. However, the protective effect of PHT was superior to HT for OO and DO, and similar to HT for LO.

Lipids and Lipolytic Enzymes of Microalgae. F. Ergan, C. Loiseau, G. Pencreach, J. Herault, and L. Poisson, Mer Molécules Santé, Université du Maine, Laval, France.

Marine microalgae are known for their levels of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids and can therefore be used as an alternative source of polyunsaturated acids (PUFAs). Among these microalgae, *Isochrysis galbana* has received increasing interest because of its high DHA content. Studies of fatty acid compositions of total lipids and lipid classes of *I. galbana* show that DHA is mainly located at the sn-2 position of the phospholipids. An enzymatic method, using commercial lipases, will be presented, leading to concentrated fractions of PUFAs. The large amount of DHA in *I.galbana* suggests the presence of lipolytic enzymes with potential interesting selectivities and substrate specificities. In order to show the presence of a new class of lipolytic enzymes, an expression plasmid was constructed by ligating the coding sequence to the plasmid vector and was validated by sequencing. In each transformant, a C-terminal histidine tag has been introduced to allow purification and immunochemical detection of the target enzyme. Results presented in this presentation show the effective functionality of plasmid construction for the recombinant protein production. Western blot identification of recombinant enzyme shows the production of a protein in the supernatant of crude extract with the expected size.

Spray Drying of Liposome Structures and Encapsulation of Black Mulberry Extract. A. Karadag[1], M. Özgüven-Gültekin[2], B. Ozcelik[2], M. Sramek[3], M. Gibis[3], R. Kohlus[3], and J. Weiss[3], [1]TUBITAK Marmara Research Center (MAM), Food Institute, Kocaeli, Turkey, [2]ITU Food Eng. Dept., Istanbul, Turkey, [3]Inst.of Food Science & Biotechnology, University of Hohenheim, Stuttgart, Germany.

Liposomes were coated with lower (LMW-C) and higher (HMW-C) molecular weight chitosan using the

layer-by-layer (LBL) depositing method. Low (DE20, LMW-MD) and high molecular weight maltodextrins (DE2, HMW-MD) were used to facilitate spray drying. Particle size and liposomal surface charges remained stable when membranes were completely covered at 0.4% chitosan. Addition of MD (20%) to uncoated liposomes caused flocculation and breakdown of the system. In contrast, chitosan-coated liposomes either slightly decreased in size due to an osmotic effect or increased in size due to aggregation depending on molecular weight of MD. Upon redispersion, all samples yielded back to original particle size, except the LMW-C coated samples that had been spray dried with HMW-MD. Mulberry extract was encapsulated in uncoated and coated liposomes and spray dried. 75% of encapsulated anthocyanin was present on the liposomal surface; chitosan coating reduced this amount (45%) and possible interactions with the ingredients in surrounding medium. Clear phase separation of uncoated liposomes mixed with MD made it unsuitable for spray drying, whereas coated liposomes retained their structure. Results suggest that appropriate design of the liquid precursor system using both adsorbing and non-adsorbing polysaccharides is crucial to produce powders that yield back dispersion similar to original liquid liposomal suspension.

PHO 2.1: General Phospholipids

Chair: S. Jadhav. Archer Daniels Midland Co., USA

Extracting Phospholipids from Egg Yolk by Green Solvents. H. Wang[1], T. Wang[2], L. Yao[2], and S. Lee[2], [1]Center for Crops Utilization Research, Iowa State University, Ames, IA, USA, [2]Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, USA.

Two types of "green" solvents were used to extract phospholipids from structurally dried egg yolk material. The effect of water content in each of the solvents on the efficiency and selectivity of the extraction was investigated.

Recovery and Quantification of Phospholipids of Oil Bodies in Sunflower Seeds. Z.M. Taher[1,2], T.J. Foster[1], I.D. Fisk[1], and D.A. Gray[1], [1]Division of Food Sciences, Sutton Bonington Campus, University of Nottingham, Loughborough, UK, [2]Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia.

Phospholipids are essential components in plant cell membranes. The high proportion of neutral lipids in oilseeds makes the recovery and quantification of phospholipids challenging, especially when the complex lipid content is very low. In this poster, a more sensitive and efficient methods of recovery, identification and quantification of the phospholipids that make up the surface of oil bodies is reported. Quantification of phospholipids in plant tissues requires a series of analytical steps like extraction of oil bodies, phospholipids recovery, chromatographic fractionation of phospholipids, its identification, and quantitative analysis of each fraction. Phospholipids fractions in this work were prepared and identified by TLC and their fatty acid profiles was analysed by Gas Chromatography Mass Spectrometry (GC-MS). It was found that the phosphatidylcholine (PC) is the most abundant phospholipid in oil bodies. An equal proportion of saturated and unsaturated fatty acids were present in the phospholipids. These results provide a further insight into the chemical nature of the surface of oil bodies and offer reliable protocol for the analysis of phospholipids associated with oil bodies.

PHO 3: Phospholipid Delivery Systems: Actives, Stability, and Antioxidant Properties

Chair: M. Rebmann, Perimondo LLC, USA

Phospholipids and Polarity Modifiers in Shape-shifting Liposomes and Their Role as Templates for Nano-construction. E. Yechiel, Elsom Research, San Antonio, TX, USA.

While nano-machines are still largely a futuristic segment of nanotechnology, they are quite common in bio-systems and are as ancient as biology itself. Though construction of nano-composites does not always require the use of nano-machines, when complex, specific-orientation, multi-component, non-planar, or asymmetric structuring is considered it may be of importance to apply such machines. What are nano-machines? Do they have common characteristics that we can study and duplicate? Can we define several common denominators that apply to natural nano-machines? There is no unequivocal answer to these questions but we can certainly identify emerging patterns from bio-systems and it may be possible to duplicate some of them and even improve upon them.

One interesting observation is that natural nano-machines in bio-systems are in some cases part of the nano-structures they maintain or construct. This means that while they are assigned a specific activity the results of such activity will also affect their well-being and their ability to continue conducting that activity.

The other observation is that, unlike regular machines that are made of many parts, nano-machines have very few components and are in many cases solid-state machines. To make all that possible, bio-membranes play a critical role in nano-machines' activity as well as in complex, specifically-oriented, multi-component, non-planar, or asymmetric structuring.

Bio-membranes are made of phospholipids as their core matrix; other components are located at specific membrane areas for functional purposes. The membrane can maintain electric potential between its two sides and as an integrating template to functional and structural proteins. In this lecture, I will discuss the unique, membrane-bound nano-machine "ATP-Synthase" which is an electro-chemical machine using the flow of protons via its core to synthesize ATP which is the common energy molecule of life.

Liposomes are a well-established bio-membrane simile, built from a phospholipids core and can be altered with various modifiers to control their physical and chemical characteristics. There are many decades of data accumulation on liposomes and they are therefore good candidates to be used in nano-structuring. The major benefit of using liposomes is the ability to use them as "Lego®-like" carriers of other molecules, whether encapsulated in their core or embedded in their membrane wall. Liposomes can be modified for charge and shape so that they can join each other in pre-determined orientation and strength and thus merge their molecular content in the desired order, orientation, and pattern. I will discuss the structuring of non-spherical or specially-shaped liposomes and shape-shifting liposomes that may contribute to creating a versatile tooling system for nano-structuring instead of the current specialized tooling system required for each type of nano-structuring, each nano-material, and each application.

Emulsion/Suspension Stability Analysis. D. Dinair, LUM Americas, Boulder, Co, USA.

In today's competitive environment, scientists, engineers, managers, are all under increasing pressure to improve existing products and introduce new ones in shorter and shorter time frames. In order to meet these challenges for dispersion based products, the correct and efficient characterization of physico-chemical properties such as stability and shelf-life parameters is therefore imperative. Traditionally, particle sizing, zeta potentials, and rheology have been used to accomplish this. As useful as they are in their own right, they nevertheless suffer from some important limitations, e.g. need for dilution, lack of accuracy, and incomplete resolution of complex systems. A newer, yet lesser known method is available that does not suffer from these shortcomings. The STEP method allows for a direct, accurate, and efficient means of dispersion characterization, particle size, stability

analysis, and shelf life prediction. The aim of this talk is to provide a short overview, present results, and provide a critical review and comparison on particle sizing (dynamic light scattering, laser diffraction, STEP), and emulsion/suspension stability characterization (zeta potentials, rheology, STEP). Data based on oil in water, water in oil, Pickering emulsions (lipid, ionic, non-ionic based), suspensions, and micro-emulsions will be presented.

Phospholipid-lecithin-based Emulsion to Stabilize Citral. Q. Huang, Rutgers University, New Brunswick NJ, USA.

Citral, a mixture of neral and geranial, is one of the most important flavor compounds used in food industry, which contributes to the fresh, lemon-like aroma of the food products. Citral can degrade rapidly by a series of cyclization and oxidation reactions to produce a variety of off-flavor compounds. Improving the stability of citral has challenged food industry for decades. Therefore, the objective of this study is to develop new strategies based on phospholipid-lecithin-based emulsions to encapsulate citral and to improve its stability.

For the encapsulation of citral, two strategies were developed to improve citral stability at acidic condition (pH = 3.0). The first strategy was to incorporate 8 different natural antioxidants (black tea extract, ascorbic acid, naringenin, tangeretin, beta-carotene, tanshinone, Co-Q10 and reduced Co-Q10) with citral together in the palm kernel fatnanoemulsions. The second strategy was to construct multilayer nanoemulsions to encapsulate citral. The multilayer emulsions were prepared by the layer-by-layer deposition technique between oppositely charged emulsion droplets and two polymer coatings: chitosan (CS) and e-polylysine(EPL). The stability of citral as well as the production of the off-flavor compounds was analyzed by solid phase microextraction gas chromatography (SPME-GC). The results suggested that encapsulation of citral in combine with the appropriate antioxidants (beta-carotene, tanshinone and black tea extract) could greatly enhance citral's chemical stability during storage; and the additional cationic chitosan interfacial layer was also effective to improve the stability of citral.

Stability of Functional Oil in Water Emulsions with Chia and Sunflower By-products. E.N. Guiotto[1,2], M.I. Capitani[2,1], S.M. Nolasco[2], and M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – (Facultad de Ciencias Exactas (FCE) UNLP – CONICET), La Plata, Buenos Aires, Argentina, [2]Facultad de Ingeniería, Dto. de Ingeniería Química (TECSE), Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Olavarría, Buenos Aires, Argentina.

The addition of fiber in food products is of a great interest in order to improve their functionality and health benefits. Oil-in-water emulsions (F=0.10) were formulated with sunflower-chia 80:20 wt/wt oil blends, chia mucilage (0.75% wt/wt) and modified sunflower lecithins (deoiled and phosphatidylcholine (PC) enriched fractions) in a range of 0.5- 2.0% wt/wt using Ultraturrax and a high-intensity ultrasound homogenizers. Optical characterization of emulsions, particle size distribution and mean diameters were determined as a function of storage time at 4° C. Mean diameters (D[3,2] and D[4,3]) of particles decreased as increasing modified sunflower lecithin concentration for both types of emulsifiers. During the storage a significant increase in these parameters was observed evolving the particle size distribution from a monomodal to a bimodal profile. All O/W emulsions showed a good stability against creaming as a function of storage for 60 d. This fact could be mainly attributed to the increase of the viscosity of the aqueous phase by the incorporation of chia mucilage (soluble dietary fiber). Chia mucilage acts as a thickener agent reducing the movility of particles of the oil phase and contributes to emulsion stability.

Effect of Antioxidant Properties of Lecithin Emulsifier on Oxidative Stability of Encapsulated Bioactive Compounds. R. Tikekar, Drexel University, Philadelphia, PA, USA.

Oxidation of encapsulated bioactive compounds in emulsions is one of the key challenges that limit shelf life of emulsion containing products. Oxidation in these emulsions is triggered by permeation of free radicals generated at the emulsion interface. The objective of this study was to evaluate the role of antioxidant properties of common emulsifiers (lecithin and Tween 20) in reducing permeation of free radicals across the emulsion interface. Radical

permeation rates were correlated with oxidative stability of a model bioactive compound (curcumin) encapsulated in these emulsions. Rate of permeation of peroxyl radicals from the aqueous phase to the oil phase of emulsion was inversely proportional to the antioxidant properties of emulsifiers. The rate of radical permeation was significantly higher ($p < 0.05$) for emulsions stabilized using Tween 20 and oxidized lecithin compared to native lecithin that showed higher antioxidant activity. Free radical permeation rate correlated with stability of curcumin in emulsions and was significantly higher ($p < 0.05$) in lecithin stabilized emulsions as compared to Tween 20 emulsions. Overall, this study demonstrates that antioxidant activity of emulsifiers significantly influences permeation of free radicals across the emulsion interface and the rate of oxidation of bioactive encapsulant.