

PHOSPHOLIPID

PHO 1: Phospholipids and Bioactive Lipids in Foods and Pharmaceutical Applications

Chairs: Ernesto Hernandez, Advanced Lipid Consultants, USA; and Samia Mezouari, Research & Consulting, Canada

Bioactive Lipids Yeonhwa Park*, *Dept. of Food Science, University of Massachusetts Amherst, USA*

Lipids are primarily known for its basic functions of energy reservoir as well as its structural components of cell membranes. However, many biologically significant functions have been discovered from 'bioactive lipids' from the basic cell biology to even cancer. Many research studies on bioactive lipids focus on the functions of fatty acids, such as n-3 fatty acids or conjugated linoleic acid, but bioactive lipids also include ceramide, diacylglycerol, eicosanoids, steroid hormones, and phosphatidic acid. Lipid soluble components including lipid-soluble vitamins and phytochemicals also contribute to improvement of health. Overview of functions of bioactive lipids in the biological system, along with its sources and potential application of these bioactive lipids to functional food product, will be presented.

Superior Antioxidant Activity of Lecithin Derived from High Oleic Soybeans Susan Knowlton*, *DuPont Company, Pioneer, USA*

Lecithin is a byproduct of vegetable oil processing and is removed during the degumming step as a mixture of phospholipids in oil. It is valued for its emulsification properties and is used in a wide variety of applications, particularly in foods. Lecithin derived from soybeans is the major form of lecithin on the market today although sunflower lecithin is gaining share because of its status as a non-GMO crop. High oleic soybeans are growing in popularity because the oil brings a soy-based solution for the food industry's

need for high stability oils. The oil is high in oleic acid and low in polyunsaturated fatty acids making it as stable as partially hydrogenated oils but without the trans fat. In addition to the intended changes in the oil, we have found that both the protein and lecithin fractions from high oleic soybeans have improved functional characteristics. The lecithin has an altered composition reflecting the oil profile and functions more effectively as an antioxidant. High oleic soybean lecithin offers the potential for a natural, clean-label, antioxidant and pan release agent.

Long-Chain Omega-3 Status in Canadian Adults: Results from the Canadian Health Measures Survey 2012-2015 Isabelle Demonty*¹, Kellie Langlois², Linda Greene-Finestone³, Rana Zoka⁴, and Loan Nguyen⁵, ¹*Nutrition Research Division, Bureau of Nutritional Sciences, Health Canada, Canada;* ²*Health Analysis Division, Statistics Canada, Canada;* ³*Public Health Agency of Canada, Canada;* ⁴*Bureau of Nutritional Sciences, Food Directorate, HPFB, Health Canada, Canada;* ⁵*Bureau of Food Surveillance and Science Integration, Food Directorate, HPFB, Health Canada, Canada*

Objective: The sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in red blood cell (RBC) membranes (Omega-3 Index) reflects long-term dietary intake and has been proposed as a marker of coronary heart disease (CHD) risk. We aimed at characterizing the Canadian population subgroups that have the lowest Omega-3 Index and may benefit from increased long-chain omega-3 intake.

Methods: Data from the Canadian Health Measures Survey Cycles 3-4, obtained from 4025 adults aged 20-79y, were pooled. Adjusted mean RBC levels of EPA, DHA, Omega-3 Index, other omega-3s, total omega-6, and ratios were calculated for different population subgroups using multiple linear regression.

Results: Only Omega-3 Index results are summarised here. Females had a higher Index (4.59) than males (4.44) ($P < 0.05$), but <3% of females and 1% of males had an Index ≥ 8 (associated with low CHD risk). The Index was higher in older adults; those 60-79y had the highest Index (4.96), and those 20-39y, the lowest (4.29). Fish intake, omega-3 supplement use, and race were the characteristics the most strongly associated with Index scores. The Index was significantly higher in participants consuming fish ≥ 2 times/week (including ≥ 1 oily fish) (5.56) versus those consuming less fish (4.37), in participants taking supplements (5.69) versus those not taking any (4.39), and in Asians (5.28) or other (pooled) ethnic groups (4.79) versus Whites (4.36) (all $P < 0.001$).

Conclusions: Despite moderate differences between subgroups, most adults have a low Omega-3 Index. Nutrition communication messages aimed at increasing long-chain omega-3 intake should target the majority of Canadians.

Lecithin as a Support System of Various

Antioxidant Management Solutions Dushka Dimitrijevic*¹, Ksenija Uroic, Simona Urbancic, Masa Hojnik Niderl², Karmen Plajsek, and Nina Janzekovic², ¹*Frutarom IFF, Slovenia*; ²*Food Protection BU of Frutarom-IFF, Slovenia*

Certain sensitive food applications, that are highly susceptible to oxidation and degradation processes due to their nature and composition, require application of different antioxidant systems in order to protect them at adequate

level. The art of combining various active ingredients is in the optimal concentration ratios, complemented with suitable aiding agents. At the same time, neither ingredients nor aiding agents should affect the flavor, odor or color of the final application. In order to meet this challenge, we have formulated synergistic antioxidant management solutions using highly potent rosemary extract solutions, tocopherols and ascorbyl palmitate, while phospholipid lecithin was added as a support system. Results showed that lecithin outperforms other tested agents in providing formulations of premium quality in terms of better solubility, lower viscosity and absence of precipitation products. Moreover, lecithin showed to be an essential ingredient in dissolving higher concentrations of ascorbyl palmitate. Such synergistic formulations exhibited better efficiency in ensuring oxidative protection of final applications, especially various oils (vegetable oils, fish oils, omega – 3 oils) and edible fats (animal fats, margarines and mayonnaises), where they provided up to 6-fold better oxidative stability of the tested samples as indicated using rancimat method. At the same time, 40% higher antioxidant efficiency was achieved in comparison to formulations without ascorbyl palmitate and lecithin. In conclusion, obtained results indicate that phospholipid lecithin has an important role in formulating synergistic antioxidant solutions and that it contributes to efficient oxidative protection of various food products.

Achieving a Tumor Docosahexaenoic Acid (DHA) Content of 5% from Different Doses and Sources of DHA Improved Chemotherapy Efficacy in Mice Bearing Patient Derived Breast Cancer Xenografts Marnie Newell*, Vera Mazurak, Lynne M. Postovit, and Catherine J. Field, *University of Alberta, Canada*

Docosahexaenoic acid (DHA) has been shown to reduce breast cancer (BC) cell growth

of immortalized cell lines in vitro and when implanted in rodents but not in more representative heterogeneous models. We sought to determine if DHA would elicit an anti-tumor effect on patient derived xenografts (PDX) and if different dietary doses (high DHA, algal source and low DHA, canola source) would elicit similar responses. Female NSG mice bearing BC PDX tumors (100 mm³) were randomized to one of three nutritionally adequate high fat diets (20% w/w \pm DHA). Treatment paradigms included a) 0% dietary DHA b) 0% DHA + TXT (docetaxel 5mg/kg) c) 4% DHA (algal source) + TXT and d) 2% DHA (DHA canola oil) + TXT. After 6 wks of chemotherapy, tumors were excised, weighed and the phospholipid (PL) composition determined. Feeding 2% or 4% DHA decreased tumor growth compared to 0% DHA \pm TXT (P

Composition and Structure of Human Milk Fat Globules: Towards Specific Interest in Infant Formula Wei Wei*¹, Mingdong Dong², and Xingguo Wang^{1,1}*Jiangnan University, China;* ²*Interdisciplinary Nanoscience Center, Aarhus University, Denmark*

Human breast milk is an oil-in-water emulsion comprising fat droplets stabilized by a complex tri-layer membrane. The surface of the milk fat globule membrane (MFGM) includes a variety of components (mainly polar lipids, proteins, neutral lipids, etc.). In recent years, this unique physical structure of MFGM are receiving increasing interests due to its nutritional effects on lipid digestion and brain development of infants. In our recent study, we determined the composition and structure of phospholipid in Chinese breast milk of different gestational age and lactation stages and infant formulas from the Chinese market. Phospholipids composition were analyzed by 31P NMR and UPLC-MS/MS (Q-ToF). Results revealed that the infant formulas have much higher phospholipid than breast milk. The highest content of phospholipid in breast milk is

sphingomyelin (> 30%), however, in infant formula is phosphatidylcholine. The structure of phospholipid on MFGM was investigated using CLSM and TEM. The mean diameter of fat globules (~200 nm) in infant formula was significantly smaller than human milk fat globules (~ 5 μ m). In breast milk, the phospholipids are evenly coverage on the triacylglycerols, while, the phospholipids in infant formula tend to aggregate. The MFGM were observed by CLSM labeled by multi-fluorescent probes. Moreover, a stable oil-in-water emulsion with large size fat globules stabilizing by nanoscale protein droplets was prepared. Further study on the effect of the unique architecture of MFGM on infant's lipid metabolism are needed to fill the gaps in the knowledge of phospholipid supplemented in infant formula.

Use of New Phospholipid Blends for Stabilization and Delivery of Bioactives in Beverages Ernesto M. Hernandez*, *Advanced Lipid Consultants, USA*

Phospholipids are widely utilized as natural emulsifiers, wetting, and dispersing agents in food as well as in many cosmetic and pharmaceutical applications. Phospholipids are also widely used in several emulsion systems because of their tendency to form structures such as bilayers, micelles, and liposomes. This has resulted in the development of new supplements and pharmaceutical products for the targeted delivery of some drugs and specialized nutrients. Applications of phospholipids have also extended into more specialized areas such as emulsion stabilization and a more efficient digestive delivery of nutrients such as essential fatty acids, and other lipids-soluble bioactives. This presentation will include the use of modified blends of soybean, canola and sunflower phospholipids in the preparation of emulsions for the delivery of omega 3 fatty acids and other bioactive lipids in

beverages. These oil-in-water emulsions were manufactured using modified blends of soybean, canola and sunflower lecithins, mono and diglycerides, and modified sugars. The main objectives of using these new emulsions are to stabilize the suspended emulsion particles by preventing creaming, coalescing and precipitation of the suspended globules and increase the bio availability of nutrients. The methods of emulsion preparation included high shear mixing and high pressure homogenization. The resulting emulsions were

analyzed for emulsion stability, organoleptic shelf life and absorption rate of bioactives. Results showed that these new lecithin blends are able to prevent creaming, coalescing and oil phase precipitation in the emulsion by balancing the specific gravity of the oil phase versus that of the water phase and also increase the bioavailability of the bioactives by the combination of phospholipids and modified sugars.

PHO 2: New Trends in Lecithin Process, Modification and Applications

Chairs: Moghis Ahmad, Jina Pharmaceuticals Inc., USA; and Swapnil Jadhav, Archer Daniels Midland Co., USA

Polar Lipids: Potentiality for Exploration

Xuebing Xu*, *Wilmar Global Research and Development Center, China*

Polar lipids exist in nature in small percentage but with large variety. Those large varieties have played various functions in life such as cell structure, delivery system, etc. Polar lipids play a big role in nutrigenomics in recent studies. This has brought interesting discussions on polar lipids in nutritional and pharmaceutical applications. With the targeted understanding and possibilities in various applications, the tailor making of polar lipids through modifications and syntheses have been received attentions, particularly with the progress of biotechnology. The artificial polar lipids have been explored for synthesis and applications in targeted cases. In general, for a lipid structure with various hydrophobic groups in various chemical structures and a hydrophilic group with the similar structural possibilities, a functional property can be tailor-made for various possibilities. This possibility can be learnt from natural polar lipids. The general possibilities will be discussed in this presentation. A few case studies will be illustrated also.

Enzymatic Production of Marine Based Lyso-phosphatidylcholine Enriched in ω -3

Polyunsaturated Fatty Acids Bo Zhou¹, Sampson Anankanbil², Yongjin He¹, and Zheng Guo*^{1,1} *Aarhus University, Denmark; ²Dept. of Engineering, Aarhus University, Denmark*

Lyso-phospholipids enriched in n-3 polyunsaturated fatty acids have both surface/interface-active functionality and health-beneficial fragments such eicosapentaenoic acid (EPA, C20:5 ω -3) and docosahexaenoic acid (DHA, C22:6 ω -3); which

could address multi-function demands in different fields. Starting with mariane phospholipids, we developed an entirely green process for enzymatic production of lyso-phosphatidylcholine. A few liquid lipases are screened for ethanolysis of purified phosphatidylcholine (PC), TLC-FID and GC-FID detections were used to monitor reaction progress and examine fatty acid compositions of different product fractions. Lipase NS-40116 from a genetic *C. antarctica* lipase A (CAL-A) displayed the best performance in either yield of lyso-PC and n-3 PUFA enrichment. Critical parameters, such as ethanol:PC ratio, reaction time and temperature, water content, and enzyme dosage are optimized. The results showed that, under optimized conditions through just one step-process, the yield of lyso-PC is up to 85% and the total n-3 PUFAs enriched up to 90% and DHA up to 75% in lyso-PC fraction. ¹H/¹³C NMR analyses were used to illustrate the reaction mechanism, which verified that the non-regioselectivity and high non-hydrolytic property towards long chain PUFAs of CAL-A are the main character to drive this high efficiency and high selective process.

Production and Applications of New Structured Phospholipids with Omega 7, Palmitoleic Fatty acid.

Ernesto M. Hernandez*, *Advanced Lipid Consultants, USA*

Palmitoleic acid (PA) is now widely marketed as a supplement and reported to have protective effects against some cardiovascular disease risk factors. PA rich foods (i.e., macadamia nuts, buckthorn seeds, and some fish oils) have been found to significantly lower levels of CRP (a measure of inflammation in the body), triglycerides, and LDL ("bad") cholesterol, while increasing HDL ("good")

cholesterol. A recently patented process also shows that interesterifying PA to phospholipids results in a product that can have superior bioactivity than the triglyceride version. In this research we synthesized and concentrated PA-ethyl esters from macadamia oil and then interesterified them into phospholipids using immobilized enzymes. We investigated the use of different lipases and phospholipases, at different reaction times and temperature parameters to study the effect on the yield of the structured phospholipids. In a typical reaction, lecithin was mixed with palmitoleic acid rich ethyl esters and immobilized lipase (or phospholipase) was added to the reaction mixture and the interesterification reaction was carried out at 40 -60 C. Enzymes Lipozyme TLIM and Phospholipase A2 were used. After the reaction was completed the immobilized enzyme was filtered off and the lecithin- oil mixture was solvent and water washed to separate the phospholipid fraction. The phospholipid fraction was dried in a vacuum oven and analyzed for palmitoleic acid incorporation. We were able to incorporate more than 40% palmitoleic acid into the phospholipid fraction. The structured phospholipid was also tested for emulsifying properties for use in food and beverage products.

Application of Zinc and Calcium Acetate to Precipitate Milk Fat Globule Membrane Components from a Dairy By-product Tao Fei, Stephanie Clark, Tong Wang, and Nathan R. Price*, *Iowa State University, USA*

There has been a great interest in developing isolated dairy lipid fractions that are rich in phospholipids (PLs) due to their health and functional properties. There is also great potential to utilize dairy by-products that are currently being discarded due to their elevated content of PLs and milk fat globular membrane (MFGM) proteins. The beta stream is an under-

utilized by-product of skim milk and anhydrous milk fat production that is currently being discarded by the dairy industry. This product contains a higher concentration of PLs and MFGM proteins but an economically feasible processing method to obtain these valuable components has yet to be obtained. Zinc acetate and calcium acetate along with mild heat treatment and pH adjustment have been shown to precipitate PLs and proteins into a pellet. This method has great potential to produce an isolated lipid fraction rich in PLs, as well as an enriched MFGM protein product when used in tandem with an ethanol extraction (90% ethanol at 70°C). The optimum precipitation treatments that were identified for the beta stream were zinc acetate at 25 mM concentration at pH of 7.5 and temperature of 30°C and calcium acetate at 100 mM concentration at pH of 7.5 and temperature of 60°C. The novel use of ethanol extraction was shown to be an effective way to remove the lipid from the precipitated pellet with a PL recovery of 97.7% for zinc acetate and 94.9% for calcium acetate under the optimum precipitation treatments.

Lecithin: The Role in Compounds (Coating, Filling), Does the Source Matter? Donna C. Studenka*, *Bunge Loders Crokiaan, USA*
Abstract not available.

Double-Layer Chia Oil Microcapsules with Sunflower Lysolecithin and Maltodextrin-Chia Mucilage Mabel Tomás*, *CIDCA (CONICET-UNLP), Argentina*

Lysolecithin obtained after enzymatic phospholipid hydrolysis exhibits interesting applications in foods. In this regard, has been reported that hydrolyzed sunflower lecithin (HSL) presents enhanced functional properties over native ones. On the other hand, the use of biopolymer combinations to improve their individual characteristics associated with the

retention and protection of microencapsulated active compounds such as omega-3 rich oils constitutes a challenge. In this context, double-layer chia oil (~64 % ALA) emulsions 5:95 wt/wt were prepared through the electrostatic deposition of chitosan (cationic character) onto hydrolyzed sunflower lecithin (anionic character) coated droplets (pH 3), microfluidization at 1000 bar with the addition of maltodextrin (Mx) or maltodextrin: chia mucilage (Mx: CM 90:10) as structural materials. The double-layer microcapsules were obtained by spray-drying of these emulsions with an efficiency of ~ 97%. The powder characterization resulted in aw and moisture contents of ~0.21-0.24 and ~1.45-1.99%, respectively. The use of the Mx: CM combination led to an increase of the D3,2 value (0.15 from 7.25 μm) and a decrease on the ζ potential (+43 to +38). Additionally, the color parameters resulted in 94.9 and 91.8, -0.1 and +0.1, 7.9 and 7.7 for L*, a* and b*, respectively. The use of HSL as emulsifying agent and the combination of biopolymers Mx-CM would be efficient in the development of chia oil multilayer microcapsules.

Forty Years of ^{31}P NMR Spectroscopy of Phospholipids Kristie Adams*¹, and Bernd W.K Diehl², ¹Steelyard Analytics, Inc., USA; ²Spectral Service AG, Germany

The power of NMR spectroscopy for studying phospholipids was first demonstrated in the 1970s. These early studies showed that ^1H and ^{13}C NMR spectroscopies had limited applicability for the study of mixtures of phospholipids, due to excessive amounts of

spectral overlap and low isotopic abundance, respectively. However, ^{31}P NMR spectroscopy was shown to be particularly powerful, due in part to the 100% isotopic abundance of the ^{31}P nucleus. Further, each unique phospholipid contains a single phosphorus atom, thus greatly simplifying spectral interpretation. Since this discovery, ^{31}P NMR spectroscopy has been used for both qualitative and quantitative determination of phospholipids in foods containing lecithin, including egg, wheat germ, soy, milk, meats and other naturally-derived phospholipid sources such as krill oil. This presentation will detail the history of phospholipid analysis by ^{31}P NMR spectroscopy: where we've been, where we are, and where we are headed in the future. A wide variety of examples will be presented, with a particular focus on the advantages of the ^{31}P NMR method, advancements in automated analysis protocols and multivariate analysis of the resulting data.

PHO-P: Phospholipid Poster Session

Chairs: Shawn Pan, Bunge North America, USA; and Sheher Mohsin, Agilent, USA

**Retardation of Anhydrous Milk Fat
Crystallization through the Addition of Dairy
Phospholipids** Zachary Cooper*¹, Casey R.
Simons², and Silvana Martini¹,¹*Utah State
University, USA; ²Utah State University, United
States*

The objective of this study was to evaluate the effect of milk phospholipids (PL) on the crystallization of anhydrous milk fat (AMF). PL were isolated from a phospholipid concentrate powder. Three mixtures of PL in AMF (0%, 0.01%, and 0.1%) were made. Samples were crystallized for 90 min at 24, 26, and 28°C. The solid fat content was measured as a function of time and fitted to the Avrami equation. Melting point, thermal behavior, viscoelastic properties, and crystal morphology were measured at 90 min and after storage at 5°C for 48 h. PL slowed crystallization as concentration increased and this effect was more pronounced at higher temperatures with no crystallization observed for the 0.1% PL at 28°C. The addition of PL resulted in bigger crystals. PL reduced G' values at 90 min for samples crystallized at 24 and 26°C with G' values of $1.8 \times 10^6 \pm 3.0 \times 10^4$ Pa, $7.1 \times 10^4 \pm 2.1 \times 10^4$ Pa respectively for the AMF and G' values of $2.1 \times 10^4 \pm 1.1 \times 10^4$ Pa and $1.4 \times 10^4 \pm 1.0 \times 10^4$ Pa for the 0.1% PL and $8.5 \times 10^4 \pm 2.9 \times 10^4$ Pa and $9.1 \times 10^4 \pm 2.1 \times 10^4$ Pa for the 0.01% PL. Similarly, hardness decreased from 20.10 ± 1.13 N for AMF at 24 and 26°C to 15.06 ± 0.77 N for 0.01% at 24 and 26°C and 0.1% at 24°C, but an increase occurred for 0.1% at 26°C 25.62 ± 1.98 N. The delay in crystallization caused by PL was also evidenced by lower enthalpy values.