H&N 1/PHO 1: Emerging Bioactives and Health Impacts

Chairs: Eileen Bailey Hall, DSM Nutritional Products, USA; and Xiaosan Wang, Jiangnan University, China

Arachidonic Acid has Anti-diabetic Actions
Gundala K. Naveen Kumar, and Undurti Das*,
BioScience Research Centre, India

Objectives: To study effect of polyunsaturated fatty acids on alloxan, streptozotocin and high fat diet induced diabetes.

Methods used: In vitro studies were done with RIN cells and in vivo studies in Wistar rats.

Results: Both ω-6 and ω-3 fatty acids prevent alloxan and streptozotocin (STZ)-induced apoptosis of rat-insulinoma (RIN) cells in vitro. Of all the unsaturated fatty acids tested, arachidonic acid (AA) prevented alloxan and STZ-induced apoptosis of RIN5F (rat insulinoma) cells in vitro and alloxan-induced type 1 diabetes and STZ-induced type 1 and type 2 DM in Wistar rats. This beneficial action of AA was not blocked by cyclo-oxygenase and lipoxygenase inhibitors. Lipoxin A4 (LXA4), an anti-inflammatory product of AA, prevented alloxan and STZ-induced apoptosis of RIN cells in vitro and type 1 and type 2 diabetes mellitus in experimental animals. AA enhanced formation of LXA4 in RIN cells in vitro and enhanced plasma LXA4 levels in alloxan and STZ-treated animals. Oral AA abrogated high-fat-STZ-induced type 2 DM in Wistar rats. AA treated HFD animals showed enhanced plasma LXA4 levels. Plasma levels of and LXA4 were decreased in patients with type 2 DM.

Conclusions: These results suggest that AA and LXA4 may function as endogenous anti-diabetic molecules.

Effects of Sesamol on Lipid Metabolism and Neurodegeneration
Xuebo Liu* and Zhigang Liu,
Northwest A&F University, China

Scope: The aim of the current study was to investigate the effect of sesamol, a natural powerful antioxidant and anti-inflammatory phenol derivative of sesame oil, on adiposity and adiposity-related metabolic disturbances in mice fed with western diet, and the potential underlying mechanisms focusing on the mitochondria-lipid metabolism. Methods & results: In the experimental model that consisted of 3-month-old C57BL/6J mice divided into 3 groups with/without sesamol in the drinking water including standard diet, high fat and high fructose diet (HFFD), and HFFD with sesamol. Results demonstrated that sesamol mitigated bodyweight gain, development of insulin resistance induced by HFFD. Sesamol was found partially normalized serum and hepatic lipid contents, as well as suppressed HFFD-induced lipogenesis in liver via regulating mitochondria-related triglyceride/cholesterol metabolism genes expressions. Importantly, sesamol decreased mass and adipocyte sizes of white adipose tissues (WATs) and brown adipose tissues (BAT) by improving mitochondria-related genes expressions including Pgc1a and Ucp1. Moreover, sesamol was also found reduced differentiation and mitochondrial metabolic inhibitors (oligomycin and antimycin A)

stimulated lipid accumulation in 3T3-L1 adipocytes. Conclusion: Taken together, this study provides compelling evidence that sesamol supplementation reduced adipocyte size and adipogenesis of diet-induced obesity by regulating mitochondria-lipid metabolism.

A Novel Method for Evaluating Anti-inflammatory Activity of Camellia Seed Oil
Ruijie Liu*[1], Niannian Lan[2], Ming Chang[1], Qingzhe Jin[1], and Xingguo Wang[1], [1]*Jiangnan University, China;* [2]*School of Food Science and Technology, Jiangnan University, China*

Introduction. Camellia seed oil has been used in Chinese traditional medicine, which is rich in oleic acid, similar to olive oil. However, little is known about the influence of Camellia oil on inflammation, and extreme water insolubility of Camellia oil greatly limits absorption efficiency and bioavailability in vitro. In order to establish a rapid method to evaluate the anti-inflammatory effect of Camellia seed oil, emulsion-based delivery systems were used. Methods. Test cases were prepared with sodium caseinate (SC), whey protein isolate (WPI), or soy protein isolate (SPI) to camellia seed oil with a ultrasonic emulsification method. LPS-induced RAW264.7 cell production of nitric oxide (NO), interleukin (IL)-6 and tumor necrosis factor (TNF-a) were determined by the kit after the treatment of test emulsion for 24h. Results. Camellia seed oil was encapsulated by SC, WPI, SPI forming nanoparticles of 323,298 and 419 nm diameter. After 24h of incubation, an emulsion stabilized with WPI showed the highest intracellular accumulation of Camellia oil (33.70±2.94 ug/mg protein),followed by that stabilized with SC (21.89±2.29) and SPI (12.69±1.87). The production of Pro-inflammatory markers showed that Camellia oil significantly inhibited the LPS-activated release of NO and inflammatory cytokines (IL-6, TNF-a) compared to controls. Anti-inflammatory effect among the three emulsions increased in the following order: SPI < SC < WPI. Conclusion. Camellia seed oil exerted anti-inflammatory effect on RAW264.7 cells stimulated by LPS. Suppression inflammation effect was highest in Camellia oil loaded emulsions stabilized by WPI proteins due to the different interfacial characteristics probably. This approach contributes to evaluate the physiological activity of lipophilic nutrients.

Dietary Krill Oil Enhances Neurocognitive Functions and Modulates Proteomic Changes in Brain Tissues of Aging Mice
Ling Zhi Cheong*, Tingting Sun, and Xiurong Su, *Ningbo University, China*

Krill are small marine crustacean with 12–50% of lipid content; they are mostly harvested in Antarctic Ocean. Krill oil contains astaxanthin; and is characterized by high concentration of long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs) mostly in the form of phospholipids (PLs) (30-65%). Due to the presence of high amount of astaxanthin and n-3 LCPUFAs, krill oil has been reported to exert positive effects on cardiovascular disease, insulin resistance and neurocognitive disorder. Despite the many studies done to show the beneficial effects of krill oil on neurocognitive function, the effects of krill oil on proteomic changes in brain tissues are rarely reported to date. Present work aimed to evaluate the effects of dietary krill oil on neurocognitive functions and proteomic changes in brain tissues of D-galactose-induced aging mice was evaluated. Dietary krill oil was found to enhance neurocognitive functions of

aging mice with significant (P<0.05) decreased in escape latency and increased in number of times crossing over the hidden platform during Morris Water Maze test. Krill oil was also found to protect against oxidative damage, lipid peroxidation and neurodegenerative diseases. Oxidative stress biomarkers of aging mice administered with krill oil showed significant (P<0.05) improvement with increased in serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels; and insignificant changes in serum malondialdehyde (MDA) level. In terms of proteomic changes, krill oil resulted in upregulation of Celsr3 and Ppp1r1b genes expression which contribute to brain development, learning and memory behavior processes.

A Brief Overview of Palmitoleic Acid, the Forgotten MUFA Gretchen Vannice*, *Organic Technologies, USA*

Little is known about cis-palmitoleic acid (16:1 n-7). Interest in this monounsaturated fatty acid (MUFA) is increasing, evidenced by more than 40 studies published in this century. Palmitoleic acid (POA) circulates in the blood with other fatty acids and is found in cell membranes and adipose tissue. POA can be consumed directly in the diet from its few food sources, mainly the oils of macadamia nuts, sea buckthorn, and some fish or it can be synthesized from the desaturation of palmitic acid via stearoyl-CoA-desaturase. US adults are estimated to consume 1–2 grams POA/day, compared to 23–29 grams/day of oleic acid (18:1n-9). POA has been described as a lipokine, biomarker, and metabolic regulator but clinical work is lacking. Cell culture and animal studies suggest that POA stimulates insulin secretion, improves glucose uptake and insulin sensitivity, increases hepatic fatty acid oxidation, enhances satiety, and reduces fatty liver. Diet studies and one clinical trial suggest improvements in blood lipid profiles and reductions in CRP with increasing POA intake. On the other hand, population studies have reported higher circulating levels of POA in adults and children, mostly notably in the obese, cardiovascular, and diabetic populations. These elevated levels may be the result of increased synthesis from excess carbohydrate and alcohol consumption. Human trials and mechanistic research on POA, the forgotten MUFA, are warranted.

Health Impact of the Newly Discovered Elovanoids: Stroke, Retinal Degenerations, Neurotrauma and Alzheimer's Disease Nicolas G. Bazan*, *LSU Health New Orleans Neuroscience Ctr, USA*

Neurodegenerative diseases and brain injury activate neuroprotective/ restorative pathways to counteract evolving adversities. The expression of these mechanisms and the mediators that signal to proteins that carry protective actions, are not well known. We have characterized a previously unknown class of mediators derived from essential omega-3 very long chain polyunsaturated fatty acids (VLC-PUFAs). VLC-PUFAs are made by ELOVL4 from DHA or EPA. ELOVL4 mutations causes certain retina degenerations, perturb brain development, trigger neuronal dysfunction, intellectual disability, and spastic quadriplegia, as well as neuroichthyotic disorders. Moreover, ELOVL4 is necessary in the skin permeability barrier for neonatal survival. The newly-identified mediators, termed elovanoids (ELVs), are di-hydroxylated derivatives of 32:6n3 and 34:6n3:

ELV-N32 and ELV-N34 respectively. Their structure and stereochemistry were established and we found them to be neuroprotective in brain and retina. ELVs enhance abundance of pro-survival (e.g., SIRT1 and Iduna) proteins in retina cells undergoing uncompensated oxidative stress. We also uncovered ELVs protection in neurons undergoing oxygen glucose deprivation, N-methyl-D-aspartate receptor-mediated excitotoxicity and ischemic stroke. Our data show neuroprotection by ELVs when administered 1 hour after 2 hours of middle cerebral artery occlusion in rats. Overall, they rescue penumbra. The signaling uncovered depicts a phosphatidylcholine that stores precursors of two mediators, DHA at sn-2, the precursor of NPD1, and C32:6n3 or C34:6n3 at sn-1 the precursors of ELVs. Thus, we disclose a new class of lipid mediators, the elovanoids, and reveal a different lipid signal bifurcation neuroprotective mechanism to sustain neural cell integrity. (Support by NIH EY005121, GM103340, and EENT Foundation).

Evidence for the Use of Docosahexaenoic Acid in the Treatment of Breast Cancer Catherine J. Field*, Newell Marnie, and Lynne M. Postovit, *University of Alberta, Canada*

Despite advances in screening, prevention, diagnosis and treatment, breast cancer (BC) remains the second leading cause of female cancer-related death, and one of the most expensive to treat. Our group and others have found that feeding sources of the omega-3 fatty acids, eicosapenataenoic and docosahexaenoic (DHA) acid are associated with a lower risk of breast cancer and feeding these fatty acids to animals prevents and reduces the growth of human and experimental mammary tumors. Women with the hardest to treat triple-negative breast cancer (TNBC) are offered neoadjuvant chemotherapy prior to breast surgery. We have found that pre-treatment of TNBC cells with DHA sensitizes these cells to the cytotoxic drug doxorubicin (DOX). We have identified potential mechanisms in vitro, including reducing proliferation, inducing cell cycle arrest and augmentation of apoptosis. More recently, we have confirmed these effects in dietary trials using two different drugs (DOX and docetaxel) and two different pre-clinical BC rodent models (nu/nu mice bearing human MBA-MD-231 cells and in a drug resistant patient-derived xenografts). We have hypothesized that these changes are the result of selective incorporation of DHA into the tumor cell membrane phospholipids affecting the function of membrane generated receptors and signals. This work supports further research on the inclusion of a supplement, with minimal to no known side effects, with current neoadjuvant therapy for BC.

Role of Oxidized Phospholipids in Myocardial Reperfusion Injury Amir Ravandi*, *University of Manitoba, Canada*

Coronary artery disease remains the leading cause of morbidity and mortality worldwide. Major advances in the treatment of acute coronary syndromes and myocardial infarction have occurred over the past 20 years. In particular, the ability to rapidly restore blood flow to the myocardium during ischemia, using percutaneous coronary interventions or thrombolytic approaches has been a major step forward. Nevertheless, while ‘reperfusion’ is a major therapeutic aim, the process of ischemia followed by reperfusion is often followed by the activation of an intense burst of reactive oxygen

species (ROS) that results in cardiac cell death and worsening myocardial function. Even with best medical care the mortality after myocardial infarction with reperfusion is at 5–10%. There are currently no therapies available for myocardial reperfusion injury. We have shown that during myocardial reperfusion injury a novel class of bioactive phospholipids, Fragmented Oxidized Phosphatidylcholines (Fragmented OxPC), are generated that result in cardiomyocyte cell death. Notably our preliminary work has identified 2 main compounds: PONPC (1-palmitoyl-2-(9-oxo)nonanoyl-sn-glycero-3-phosphocholine) and POVPC (1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine), that are the most abundant OxPC generated during I/R. We have also shown that exposure of cardiomyocytes to PONPC and POVPC, results in cell death through a mitochondrial related pathway, resulting in loss of mitochondrial membrane potential and loss of mitochondrial function. Our data strongly suggests that fragmented OxPCs are potent inducer of cardiomyocyte cell death during I/R. If this view is correct then this will establish a novel therapeutic pathway to avert cell death during I/R injury by inhibiting the activities of OxPCs.

PHO 2: Chemical and Biochemical Advancement in the Phospholipid Field

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

Phospholipids Modification with Enzymes: A Re-visit Xuebing Xu*, *Wilmar Global Research and Development Center, China*

Abstract not available.

Phenolipids for Delivery Systems: Synthesis and Characterization Sampson Anankanbil*[1], Bianca Perez[1], Chiranjib Banerjee[2], Katarzyna Widzisz[2], and Zheng Guo[2], [1]*Dept. of Engineering, Aarhus University, Denmark;* [2]*Aarhus University, Denmark*

Structured phospholipids (PLs) are essential molecules for cross-disciplinary applications. Therefore, the development of new synthetic routes for structural modification of PLs has generated interest among chemists. Structural modification of PLs with phenolic acids yield phenolipids, which can confer both physical and oxidative stability to highly sensitive lipophilic bioactives in delivery systems. We report a two-step facile, mild and scalable synthetic approach for the synthesis of a novel array of phenolipids with varied fatty acyl chains (12, 14, 16 & 18). Catalyst- and solvent-free modification of glycerophosphatidylcholine (GPC), and acylation with phenolic acids yielded the desired compounds. Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR) and Langmuir-Blodgett analysis characterized the phenolipids. Furthermore, the phenolipids were evaluated for both in vitro and in vivo antioxidant potentials, as well as for surface activity by fluorescence microscopy and dynamic light scattering techniques. Phenolipids and unmodified PLs with fatty acid chain lengths of 16 and above adopted orthorhombic packing while those with <16 fatty acyl units adopted hexagonal packing modes. The antioxidant potentials of the new phenolipids were comparable to free phenolic acids and significantly ($p<.05$) improved compared to unmodified soybean lecithin. In addition, the surface activity and emulsification properties of the phenolipids were superior to unmodified soybean lecithin. In conclusion, this is the first study, using GPC as starting material, to synthesize structured PLs containing phenolic acids under mild conditions. The resulting phenolipids have superior/added functionalities compared to unmodified PLs and free phenolic acids or their admixtures. The new phenolipids can find applications in food, cosmetics and pharmaceutical formulations.

New Approaches in Non-aqueous Enzymology for Modification of Lipids and Phospholipids Douglas G. Hayes*, *University of Tennessee, USA*

This lecture will review the fundamentals of conducting enzyme-catalyzed transformations in nonaqueous media, particularly, organic solvents, focusing upon the modification of lipids and phospholipids by lipases and phospholipases and newer developments. Topics to be covered include preparation of the enzyme and reaction medium, control of water activity, and guidelines for conducting the enzymatic reactions. Also, guidelines for the use of ionic liquids and deep eutectic solvents as reaction media will be provided.

Ability of Soy Lecithin Oleogel Emulsions to Protect Probiotics and Prevent Oxidation Nicole I. Gaudino, Stephanie Clark, and Nuria C. Acevedo, *Iowa State University, USA*

Phospholipids (PL) have been shown to bind to probiotics and increase their viability. However, phospholipids are readily prone to oxidation, which hinders their use as probiotic protectants dairy products. The objective of this study was to produce a soy lecithin: stearic acid oleogel emulsion (LOGE) containing Lactobacillus acidophilus and Bifidobacterium lactis in the aqueous phase with the aim to improve their viability while decreasing PL oxidation. Probiotics were inoculated into a LOGE with 20% solids (1:1 soy lecithin: stearic acid) and 10% water. LOGE were stored at 4C aerobically, and plate counts were conducted for six weeks to determine the viability of the probiotics. The counts were compared to those of four controls: canola oil, a stearic acid oleogel emulsion, MRS broth, and MRS broth supplemented with soy lecithin. Oxidative stability of the oleogel emulsions were determined through measurement of peroxide and p-anisidine value upon 30, 45, and 60 days storage at room temperature. The physical and microstructural characteristics of the LOGE reduced the oxidation of lecithin by preventing interaction between lecithin and radical species. The gel matrix protected the probiotics from oxygen, acidity, and other factors that are detrimental to probiotic growth. Through reducing the oxidation of phospholipids and increasing the survival of probiotics, oleogel emulsions can be incorporated into yogurt and other dairy products to enhance the delivery of probiotics.

Lipids as Mediators of Cardiomyocyte Cell Death During Ischemia/Reperfusion Injury Aleksandra Stamenkovic*[1], Kimberley A. O'Hara[2], David C. Nelson[2], Andrea L. Edel[2], Grant N. Pierce[2], and Amir Ravandi[1], [1]*University of Manitoba, Canada;* [2]*Institute of Cardiovascular Sciences, Canada*

Reperfusion represents the major treatment for myocardial infarction. However, it leads to generation of reactive oxygen species (ROS) that can produce oxidized phosphatidylcholines. Two fragmented ones, POVPC (1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine) and PONPC (1-palmitoyl-2-(9'-oxo-nonanoyl)-sn-glycero-3-phosphocholine), that are highly generated in I/R, significantly decrease cardiomyocyte cell viability in vitro. However, the mechanism of cardiotoxic action of oxPCs remains unknown. Ferroptosis is an iron dependent form of cell death, induced by accumulation of lipid oxidation products. Therefore, we wanted to examine the role of ferroptosis in oxPC mediated cell death. In adult rat ventricular cardiomyocytes, an inhibitor of ferroptosis, Ferrostatin -1 was tested together with POVPC. Cells were exposed to 1 h ischemia followed by 1 h of reperfusion, and, ferrostatin-1, was added at the time of reperfusion. Fura-2 AM was used to measure Ca transients in cells treated with PSPC and POVPC. Video edge detection was used to measure contractile performance. Two fragmented OxPCs, POVPC and PONPC, were cardiotoxic in a concentration dependent manner from 0.1µM-10µM. Treatment of cardiomyocytes with ferrostatin-1, added at the same time as oxPC, attenuated POVPC-induced cell death, maintaining viability at 75% (p: Oxidized phospholipids, generated in

I/R are potent mediators of cardiomyocyte cell death through the ferroptotic pathway. These results may lead to a new therapeutic approach to prevent cell loss during I/R injury which currently does not exist.

Sunflower and Soy Bean Lecithin: Interfacial Rheology and Kinetics at the Oil / Water Interface plus the Influence of Counter Ions
Arnulf Schoeppe* and Prashandh Sankarappan, *Cargill Texturizing Solutions Deutschland GmbH & Co.KG, Germany*

Sunflower and soy bean lecithins are two important low molecular weight emulsifiers in food industry often interchanged by one another. For the study well analysed liquid and de-oiled matching pairs of sunflower and soy bean lecithin were produced. The behavior at the oil / water interface of these emulsifiers were studied in sunflower and soy bean oil by profile analysis tensiometry. Both the sunflower and the soy bean lecithin were mixed with the respective oils in liquid and in de-oiled form. Interestingly the kinetics of the interface active molecules of the de-oiled lecithins was much faster compared to the liquid ones though their concentration and composition was the same. All curves were fitted to a single exponential decay. Adding sodium and calcium salts to the aqueous phase led to a faster lowering of the interfacial tension compared to no added salt. This indicates that the charged phospholipids in the lecithin are influenced by the charge of the cations. The complex elastic modulus of the interface showed only a limited decrease regardless of the added salt. This shows that neither the monovalent sodium nor the divalent calcium cations were able to strongly influence the mechanical properties of the formed interface.

Development of Phospholipid-Enriched Oleogels and Oleogel Emulsions Edible Semisolid Applications
Nicole I. Gaudino[1], Saeed Mirzaee Ghazani[2], Alejandro G. Marangoni[2], Stephanie Clark[1], and Nuria C. Acevedo[1], *[1]Iowa State Unviersity, USA; [2]University of Guelph, Canada*

Soy lecithin can form an oleogel in the presence of a small quantity of water. To date, no soy lecithin based oleogel emulsion has been developed. The objective of this study was to characterize a novel soy lecithin based oleogel (LOG) and oleogel emulsion (LOGE) prepared with different proportions of stearic acid. Oleogels were developed with 1% (w/w) of water, and two gelator concentrations (20% and 30%) with soy lecithin: stearic acid ratios (0:10, 3:7, 5:5, 7:3, 10:0). The same proportions were used to prepare LOGEs with 10% and 20% (w/w) of water. The hardness of the samples was analyzed using a Texture Analyzer and the thermal properties with a Differential Scanning Calorimeter. Small (SAX) and wide (WAX) angle x-ray diffraction studies were conducted to determine the nanostructure of the samples. The results indicate that the LOGs were formed through the entanglement of reverse wormlike micelles of lecithin; however, the addition of water from 1 to 10% to form LOGE altered the matrix structure. Stearic acid stabilized the LOGEs, creating a steric acid crystalline network in conjunction with the wormlike reverse micelles. Therefore, the LOGEs were significantly harder and exhibited higher melting and crystallization temperatures than the LOGs. A synergistic effect between the soy lecithin and stearic acid was observed, since there was a significant increase in hardness. The novel LOGEs with stearic acid can be used as a more stable

alternative to soy lecithin oleogels when the objective is to achieve semi-solid characteristics.

Achieving a Docosahexaenoic Acid Content of 7% Improved the Efficacy of Chemotherapy in Mice Bearing a Triple Negative Breast Cancer Human Xenograft Marnie Newell*, Vera Mazurak, Lynne M. Postovit, and Catherine J. Field, *University of Alberta, Canada*

It is well established that docosahexaenoic acid (DHA), reduces breast cancer cell growth of human tumor cells in vitro and when implanted in rodents. Because immortalized cell lines do not represent the heterogenicity seen in human tumors, we sought to confirm this work using patient derived breast cancer xenografts (PDX). NSG mice (6-week-old female) bearing subcutaneous triple negative PDX tumors (100 mm3) were randomized to one of two nutritionally adequate high fat diets (20% w/w ±5% DHA). Half the animals in each group were treated twice weekly with docetaxel (TXT, 5mg/kg; intraperitoneal) for 6 weeks (n=7 per group). Feeding DHA in combination with TXT resulted in significantly smaller tumors (2.1 ± 0.4g vs. 5.8±0.8g). The content of DHA in plasma phospholipids (6.99±0.1%) and tumor phospholipids (5.2 ± 0.2%) was higher as expected than the control TXT diet mice but also higher than that of the tumor bearing mice fed DHA (without TXT) ($p<0.05$). Immuno-histochemical staining of Ki67, CD95 and TUNEL confirmed lower proliferation and increased apoptosis in the DHA TXT tumors ($p<0.05$). Protein analysis confirmed changed expression of proteins involved in apoptosis including BCL2, Caspase 3, Caspase 7 and PARP in DHA TXT tumours ($p<0.05$). This study confirms, for the first time in patient derived xenografts, that feeding a diet supplemented with DHA facilitates the anti-cancer effect of TXT on breast cancer cells and our work suggests a target plasma lipid concentration associated with an effective concentration in tumor membranes that facilitates reduced proliferation and increased apoptosis. (Supported by CIHR.)

PHO 3: Developments and Applications of Novel and Modified Phospholipids

Chairs: Ernesto Hernandez, Advanced Lipid Consultants, USA; and Xuebing Xu, Wilmar Global Research and Development Center, China

Preparation and Functional Evaluation of Antarctic Krill Lipid Yuanfa Liu[1], Dewei Sun[2], Peirang Cao[1], and Zong Meng[3], *[1]Jiangnan University, China; [2]School of Food Science and Technology, Jiangnan University, China; [3]School of Food Science and Technology, Jiangnan University, China*

As Antarctic krill is a highly abundant species and a huge bio-resource for human beings, proper technology development is serious needed for exploration as nutrient supplementation for food industry. This study had developed a novel procedure, combination heat pump drying and freeze-drying for Antarctic krill with energy efficiency, and then subcritical n-butane extraction of Antarctic krill oil (KO) with higher lipid recovery and better quality. This procedure could be applied in a large scale for food industry. KO has high EPA and DHA-containing phospholipids, which might be better bioavailability with some uncertainty. KO has a significant amount of astaxanthin, an important natural antioxidative component. Recently, KO seems to be valuable marine food supplementation but there is still no concrete conclusive on clinical efficacy in the management of chronic metabolic diseases. Since different technologies could greatly influence oil properties and healthy effect as well, the aim of this study was to investigate the effects of KO, prepared by combined dry and then subcritical extraction, on C57BL/6J mice with experimentally induced obesity and induced damaged by with polar compounds generated from deep-frying process of palm oil, respectively. The mice fed with the diets containing the KO, in contrast to the mice fed with normal chow and containing polar compounds, reduced animal body weight gain and improved dyslipidemia, glucose metabolism as well as oxidative damaged to some extent. The results indicated that the KO used in this study could be beneficial food supplements to animal health.

Review of Uses of Phospholipids in Delivery Systems and Bioactive Carriers Ernesto Hernandez*, *Advanced Lipid Consultants, USA*

Phospholipids are widely utilized as natural emulsifiers, wetting, and dispersing agents in food applications. They are also reliable ingredients, excipients or carriers in many pharmaceutical and cosmetic formulations. Their properties to form structures such as bilayers, micelles, and liposomes allow for many applications in pharmaceutical, food, and cosmetic products. This has resulted in the developments of new pharmaceutical products such specialized drug delivery systems. Applications of specialty phospholipids have extended into more specialized pharmaceutical and cosmetic products such as transdermal carriers and skin emolliency agents. This presentation will review specific targeted applications of phospholipids in pharma and cosmetic fields including the manufacture of lipid vesicles with entrapped bioactive elements able to partition into the skin layers and deliver drugs and bioactive transdermally as well as delivery of drugs intravenously, orally, and parenterally. Presentation will also focus on uses in excipients

to facilitate delivery of bioactive compounds, to act as fillers, binders, lubricants, solubilizers, emulsifiers and emollients in a variety of delivery forms including tablets, capsules, emulsions, ointments, creams and lotions. Examples of other non-direct applications will include gene delivery, diagnostic imaging and, medical devices.

Composition and Structure of Phospholipid in Breast Milk: Towards Specific Interest in Infant Formula Wei Wei*[1], Mingdong Dong[2], and Xingguo Wang[1], [1]*Jiangnan University, China;* [2]*Interdisciplinary Nanoscience Center, Aarhus University, Denmark*

Phospholipid in breast milk showed beneficial effects on gut and neuro-development in early infancy. In some infant formula, phospholipids are added as emulsifier (mainly lecithin) or milk fat globule membrane (MFGM) product. Understanding the difference of phospholipid in breast milk and infant formula is of both scientific and industrial significance. In our recent study, we determined the composition and structure of phospholipid in Chinese breast milk and infant formula from Chinese market. Phospholipid and fatty acid (FA) composition were analysed by ^{31}P NMR and gas chromatography, respectively. Results revealed that 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 phospholipid FA composition of breast milk and infant formula are significantly different, especially some essential FAs. Moreover, the structure of phospholipid on MFGM were investigated using Confocal and TEM. The mean diameter of fat globules (~200 nm) in infant formula was significantly smaller than human milk fat globules (~ 5 μm). In breast milk, the phospholipids are evenly coverage on the triacylglycerols. However, the phospholipid in infant formula tend to aggregate. The phospholipid packing on MFGM were observed by Confocal labelled by multi-fluorescent probes, which indicated no liquid-ordered domains (so called lipid raft) were observed. Further study on the effect of the unique architecture of MFGM on infant's lipid digestion are needed to fill the gaps in the knowledge of phospholipid supplemented in infant formula.

Design of Two-layered Microcapsules of Chia Oil by Using Sunflower PC-enriched Lecithin and the LBL Technique Luciana M. Julio[1], Claudia N. Copado[1], Vanesa Y. Ixtaina[1], Bernd W.K. Diehl[2], and Mabel Tomás*[1], [1]*CIDCA (CONICET-UNLP), Argentina;* [2]*Spectral Service AG, Germany*

Microencapsulation constitutes a strategic technology to protect and deliver sensitive bioactive lipids as ω-3 PUFAs. One and two-layered chia oil (~64% α-linolenic acid) emulsions were prepared by the layer-by-layer electrostatic deposition technique (600bar,pH3), using sunflower phosphatidylcholine-enriched lecithin, maltodextrin and chitosan as wall material. From these emulsions, the corresponding one and two-layered microcapsules were produced by freeze-drying and stored at HR33%, 25±2ºC for 120d. Microcapsules presented aw and moisture contents of ~0.15 and ~0.09%, respectively. The microencapsulation efficiency was 85.8 and 89.3% for one and two-layered systems. Loading and loading-efficiency for oil and ω-3 resulted in 19.23, [1]5.9, [1]1.97 and 10.74 for mono and 19.08, [1]6.9, [1]1.88 and 10.57 for bilayer powders. A remarkable difference was detected between the induction time and the remaining of ω-3 PUFAs

after 50d of mono (12.6h,8.9%) and bilayer (22h,82.1%) systems. Thus, two-layer microcapsules with sunflower phosphatidyl-choline-enriched lecithin proved to be efficient delivery systems of ω-3 PUFAs from chia oil with potential application in the food industry.

Low Molecular Weight Food Grade Emulsifiers including Soy Lecithins / Phosphatidylcholine span a wide Range of Interfacial Tensions and Interfacial Rheological Properties Arnulf Schoeppe*, and Padmavathi Sridharan, *Cargill Texturizing Solutions Deutschland GmbH & Co.KG, Germany*

The interfacial rheology and interfacial tension of a series of low molecular food grade emulsifiers was investigated by profile analysis tensiometry. Emulsifiers tested were mono-diglycerides, polyglycerine-polyricinoelate, sorbitan monoleate, and a series of lecithins phospholipids: de-oiled soy lecithin, highly enriched soy phosphatidylcholine and PC depleted soy lecithins. The emulsifiers span a range of different properties regarding their use for o/w and w/ o emulsions their potential to lower the interfacial tension and influence the interfacial rheological parameters of an oil / water interface. It turned out that the interfacial properties of the lecithins / phosphatidylcholine were strongly dependent on the respective phosphatidylcholine content. The oil / water interface covered with highly enriched soy phosphatidylcholine shows a very effective lowering of the interfacial tension and a very low elastic modulus while the phosphatidylcholine depleted fraction was able to lower the interfacial tension only to a lesser extent but in contrast to these showing a much higher elastic value of the interface. This might be related finally to emulsion properties.

Extraction, Purification and Enzymatic Modification of Phospholipids from Antarctic Krill Shulai Liu*, Jie Hu, Kaixi Xu, Bokai Yu, and Yuting Ding, *Zhejiang University of Technology, Chin*

There is significant commercial interest in phospholipids preparation from Antarctic krill because it is rich in omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3) acids. Supercritical CO2 fluid (SC-CO2) was used to extract neutral triglycerids and astaxanthin from krill powder. Temperature, pressure, time and the flow of ethanol during the extration process was optimized by response surface methodology. Phospholipids was furtherly extracted by SC-CO2 with ethanol from the residual degreased krill power. PI and PC was purified by the ethanol with ammonia water and column chromatography. Lysophospholipids enriched Omega-3 fatty acids was synthesized by phospholipase A2 catalyzed hydrolysis. The mixture of phospholipids contains 51.49±1.07% of phosphatidylcholine (PC), 11.41±1.21% of phosphatidylethanolamine (PE) and 5.21±0.57% of phosphatidylinositol (PI). The content and recovery rate of PI is 62.13±1.07% and 94.21±1.17%. The purity of PI an PC was 62.13±1.07% and 95.18±1.17%.

Novel Structured Phospholipids and Applications from Avocado Oil Sara KoohiKamali* and Ernesto Hernandez, *Advanced Lipids Consultants, USA*

Avocado lipids are relatively rich in phospholipids; however, studies on composition and emulsifying capacity of phospholipids from

avocado oil are scarce. The objective of this research was to investigate and compare the fatty acid content, phospholipid composition, and emulsifying properties of lecithin recovered from water degumming (WDA) and enzymatic degumming of Avocado oil (EDA). The soybean lecithin was employed as the control to compare Avocado oil emulsifying properties with commercial soybean lecithin as the control (WDS). Synthesis of structured lysophospholipids containing fatty acids in sn-2 position using phospholipase A1 (PLA1) was also applied.

Fatty acid composition and polar lipids of the EDA, WDA and WDS samples determined using GC-FID and HPLC-ELSD. The major phospholipids found in avocado lecithin were phosphatidylethanolamine (PE, 55%), lyso-phosphatidylethanolamine followed by phosphatidylcholine (PC, 20%), phosphatidylinositol (PI, 15%) and lysophosphatidylcholine (LPC).

Oil-in-water (O/W) emulsions were prepared using phospholipids EDA, WDA and WDS as emulsifiers, commercial soy oil as the oil phase, and deionized water at pH 7.5 as the aqueous phase. O/W emulsions were prepared at several water to oil ratios 1:9-4:6) and emulsions were evaluated for sedimentation, creaming patterns, particle size distribution and microscopic imaging of emulsion droplets.

Both type of EDA and WDA lecithin from Avocado oil indicated to be rich in PE or cephaline which has an important role in nervous tissue such as the white matter of brain, nerves, neural tissue, and in spinal cord. In addition, both EDA and WDA lecithins indicated to have good oxidative stabilities due to their high content of monoenoic fatty acids (i.e., oleic and palmitoleic).

This study provided a better understanding of the some of the properties of phospholipids, enzymatically modified and non-modified, from avocado oil. These structured phospholipids are a promising source of PE-rich emulsifier and nutritional ingredient.

Characterization of Novel Vegetable Lecithin and its Application in Emulsions Xuebing Xu, Fang Cong*, and Binbin Chen, *Wilmar Global Research and Development Center, China*

Palm lecithin, rice bran lecithin and sunflower lecithin have not been well used for various reasons for now. High value-added lecithin by-products may be disposed as waste in oil processing factories, causing environmental problems and economic waste. To make better use of lecithin resources, this study will review the phospholipid composition and side chain fatty acids composition in lecithin from palm, rice bran and sunflower. The recovery methods from different feedstock will also be discussed. Meanwhile, the powdered lecithin from rice bran and sunflower will be prepared and characterized for emulsion evaluation. Firstly, through water degumming, we prepared crude rice bran lecithin and sunflower lecithin with crude oil as feedstock, and then removed most of wax by hexane precipitation, followed by removing triglyceride and fatty acid by acetone precipitation. The emulsion with rice bran lecithin and sunflower lecithin as emulsifier was prepared respectively through shear and homogenization process, and the stability of the emulsion was analyzed and evaluated by means of stability analyzer, laser particle size and electric potential etc.

PHO-P: Phospholipid Poster Session

Chair: Ernesto Hernandez, Advanced Lipid Consultants, USA

1. **Physical, Oxidative Stability and Microstructure Characteristics of Structured Lipid/Skim Milk Emulsions Prepared by using Different Emulsifiers** Abdelmoneim H. Ali[1*], Wei Wei[2], Sherif M. Abed[2], Sameh Korma[1], Qingzhe Jin[3], and Xingguo Wang[3], *[1]School of Food Science and Technology, Jiangnan University, China, China; [2]State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, School of Food Science and Technology, China; [3]Jiangnan University, China*

Recent research activities have increasingly focused on the development of invented structures to protect bioactive components and nutrients. Widespread transfer of basic knowledge about the production and stabilization of double emulsion systems to the food sector has been occurred in the last decade. In this study, a novel structured lipid/milk emulsion was prepared by using different types of emulsifiers (including milk phospholipid, egg yolk phospholipid, soy bean lecithin, sodium dodecyl sulfate, or Triton X-100). Medium chain triacylglycerols and docosahexaenoic-single cell oil were used to prepare the structured lipid by using lipase-catalyzed interesterification in a solvent free system. The major fatty acids detected in the synthesized structured lipid were caprylic (40.99%), capric (20.60%), docosahexaenoic (19.45%), palmitic (12.23%), and docosapentaenoic (4.10%) acids. The emulsions were stored at room temperature, and the physical, microstructure, and oxidative stability characteristics were studied. The oxidative stability of emulsions formulated by using milk phospholipid followed by those formulated by using soybean lecithin were better than emulsions formulated by using egg yolk phospholipids or synthetic surfactants. We recommend further studies to investigate the potential applications of that novel emulsion in yogurt and infant formulas supplementation. As well, the nutritional characteristics of the emulsion should be evaluated.

2. **Effect of Modified Sunflower Lecithins on Bread Quality.** Estefania N. Guiotto[1], Mabel Tomás[2], and Claudia M. Haros[3], *[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Argentina; [2]CIDCA (CONICET-UNLP), Argentina; [3]Grupo de cereales, Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Spain*

Lecithins are a mixture of acetone-insoluble phospholipids and other minor substances. The most common processes used for their modification are fractionation to produce enriched fractions in specific phospholipids or introduce enzymes to obtain lysolecithins. The objective of the present work was to determine the effect of the addition of modified sunflower lecithins (phosphatidylcholine enriched fractions, lysolecithins) and soy lecithin on the bread quality. Each emulsifier was tested at two levels 0.5 and 1.0%. Bread quality attributes such as crust color, specific loaf volume, and firmness were measured. The crust color values (62.5–64.3) did not change as an effect of adding emulsifiers. The addition of different lecithins improved the bread quality comparing to control bread, differences in the specific loaf volume and firmness between control and bread samples

with emulsifiers were observed ($p<0.05$). The bread formulated with hydrolyzed sunflower lecithin at 1.0% level exhibited the lowest firmness and the highest specific volume values. The modified lecithin sunflower ingredient could be used as an improver in bakery products.

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3. **Preparation of Phosphatidylcholine by Transphosphatidylation of Phosphatidylethanolamine and Phospholipase D** Wei-Ju Lee*, *Taipei Medical University, Taiwan*

Phosphatidylethanolamine (PE) is one of the major phospholipids in soybean lecithin, which is only lesser than phosphatidylcholine (PC). In this study, an enzymatic method was established to extend the PC portion by means of transphosphatidylation to convert PE to PC for the increasing demand of high-purity PC in industries. PC and PE were separated from soybean lecithin (95% ethanol-soluble fraction) by silica gel chromatography with content of 86.4% and 90.2%, and recovery of 71.8% and 80.3%, respectively. Phospholipase D (PLD) from Streptomycese sp. possessed better transphosphatidylization ability to convert PE to PC than PLD from cabbage. PLD from Streptomycese sp. was immobilized by covalent binding to glutaraldehyde-activated magnetic particles. The optimum pH value and temperature of immobilized PLD were pH 7.0 and 37°C, respectively. Reaction parameters of substrate and calcium ion concentrations were investigated to determine optimum conditions. The PC yield of the reaction product was 64.9%, achieved at 12 hours in the ethyl acetate/phosphate buffer (pH 7.0) system containing 120 mM Ca2+, 2.5 M choline chloride and 1 unit (U) of immobilized PLD at 37°C. The immobilized PLD could be repeatedly used for ten times and showed no significant activity loss. The PC molecular species compositions of synthesized PC were different from the origin PC in soybean lecithin. The synthesized PC was mainly composed of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC) and 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (LLPC), which were accounting for 72.4%.

4. **Release of ω-3 and ω-6 Epoxides from Acidic Phospholipids of Lipoproteins by IIA, V and X Secretory Phospholipases A2** Arnis Kuksis* and Waldemar Pruzanski, *University of Toronto, Canada*

Cytochrome P450 epoxygenases convert arachidonic acid (ω-6) to epoxyeicosatrienoic acid (EET) and eicosapentaenoic acid (ω-3) to epoxyeicosatetraenoic acid (EEQ), but docosahexaenoic acid (ω-3) to epoxydocosapentaenoic acid (EDP) bound to glycerophospholipids, which are also formed during autoxidation. The ω-3 and ω-6 epoxides show opposite biological effects, when released as free fatty acids. We had previously shown that the epoxides of plasma lipoprotein PtdCho are hydrolyzed at different rates by group IIA, V and X secretory PLA2s and now demonstrate further differences in the hydrolysis of lipoprotein PtdEtn, PtdIns and PtdSer by the above enzymes. The enzymatic hydrolyses were conducted as described earlier. The PtdEtn epoxides were determined as hydrogen adducts, while the epoxides of PtdIns and PtdSer were done as the monosodium and disodium adducts, respectively, all in positive ion current. The amounts of the

epoxides ranged from high picograms to low nanograms/mg protein. Our study demonstrates that the rates of epoxide hydrolysis vary markedly with the fatty acid series and the glycerophospholipid source of the epoxide, as well as the sPLA2 group. Assuming that these in vitro studies represent in vivo conditions, it is concluded that the biological activity of the epoxides (angiogenesis, tumor growth and metastasis) may vary with the source of the epoxide and the activity and specificity of the phospholipase involved in their release.