2009 Annual Meeting Abstracts

Phospholipids

MONDAY

MORNING

PHO 1: Bioactive Lipids I

Chair(s): M. Ahmad, Jina Pharmaceuticals Inc., USA; and B. Sebree, Archer Daniels Midland Co., USA

Synthesis and Properties of Novel Bioactive Sphingophospholipids. R. Bittman, City University of New York, Queens College and The Graduate School, Flushing. NY, USA

The syntheses and properties of novel bioactive phospholipid analogs will be discussed. Included in the lecture will be: analogs of alkenyl-acyl phospholipids (plasmalogens) that exhibit antioxidant properties; phosphonate analogs of the immunosuppresant lipid FTY720, which acts on sphingosine 1-phosphate receptors; caged analogs of sphingosine 1-phosphate that contain coumarin and quinoline cages; carbamate analogs of the antitumor ether phospholipid ET-18-OCH3 that display a selective cytotoxicity for prostate tumor cells.

Synthesis of Methoxylated Alkylglycerols Present in Shark Liver Oil. C.D. Magnusson, G.G. Haraldsson, Science Institute, University of Iceland, Reykjavik, Iceland

Ether lipids of the 1-O-alkyl-sn-glycerol type occur widely in nature but are especially abundant in the liver oil of sharks and other elasmobranch fish. They have shown various beneficial effects on human health. Methoxylated alkylglycerols possess a methoxyl group located at the second position of the 1-O-alkyl chain and they constitute 0.3 ? 4% of the total amount of the alkylgycerols in shark liver oil. The principal types of the methoxylated alkyl chains include two saturated (C16:0 and C18:0), two monounsaturated (C16:1 and C18:1) with a double bond at the fourth position of the 1-O-alkyl chain and a polyunsaturated (C22:6) one. Methoxylated alkylglycerols have shown immune stimulating effects and anticarcinogenic properties. The current work describes the total synthesis of enantiomerically pure (Z)-(2´R)-1-O-(2´-methoxyhexadec-4´-enyl)-sn-glycerol which to the best of our knowledge has not been synthesized before. All intermediates and the final product were fully characterized by traditional organic synthesis analytical methods including 1H and 13C NMR and IR spectroscopy and high-resolution mass spectrometry. Moreover, the absolute configuration and optical activities were determined for the final product and intermediates involved.

Recent Developments in Bioactive Sponge Phospholipid Fatty Acids - An Overview. Nestor M. Carballeira, Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, Rio Piedras, Puerto Rico, USA

Our research group has been investigating the unusual symmetrical phospholipids from Caribbean sponges. These phospholipid mixtures contain unusual phospholipids with unprecedented fatty acids displaying interesting biological properties. For example, we have shown that the very-long chain Δ5,9 fatty acids are very good inhibitors of the enoyl-reductase (FabI) from Plasmodium falciparum, the key parasite responsible for malaria. Other research groups have also shown that the very-long chain Δ5,9 fatty acids are also good inhibitors of the human topoisomerase I, thus implying that these fatty acids might have potential as anticancer agents. Another studied class of sponge phospholipid fatty acids is the α-methoxylated fatty acids. These α-methoxylated fatty acids also occur in unusual phospholipids and we have demonstrated that the methoxylated fatty acids display enhanced antifungal and antileukemic properties when compared to the corresponding non-methoxylated fatty acids. Therefore, the value of α-methoxylation as a biomedical tool to enhance the antifungal and/or biomedical properties of fatty acids will be postulated. The characterization, synthesis, antimalarial, antifungal, and enzyme inhibitory properties of these unusual phospholipid fatty acids will be presented and discussed.

Chemoenzymatic Synthesis of Structured Phosphatidylcholine Positionally Labeled with Pure EPA and DHA. B. Kristinsson, C.D. Magnusson, G.G. Haraldsson, University of Iceland, Science Institute, University of Iceland,

Reykjavik, Iceland

We have previously described the synthesis of structured MLM type triacylglycerols possessing pure saturated medium chain fatty acids (MCFA, C6 - C12) at the terminal positions and pure EPA or DHA at the mid position. This was accomplished by a highly efficient two-step chemoenzymatic process. An immobilized Candida antarctica lipase (CAL) was observed to display excellent regioselectivity toward the end positions of glycerol using vinyl esters as acylating agents. The n-3 fatty acids were subsequently introduced to the remaining mid position using EDAC as a chemical coupling agent. The current work describes a related chemoenzymatic synthesis of enantiopure structured phosphatidylcholine (PC) possessing a pure MCFA at the sn-1-position and pure EPA or DHA located at the sn-2-position of the glycerol backbone. This was accomplished by a two step chemoenzymatic process starting from optically pure sn-glycerol-3-phosphatidylcholine (GPC). In the first step GPC was acylated exclusively at the sn-1 position using lipase and vinyl esters of pure MCFA (C6 - C12) to afford regiopure 1-acyl-sn-glycero-3-phosphocholines (2-lysophosphatidylcholine, 2-LPC) in very high purity and yields. The 2-LPC adducts were subsequently treated with pure EPA and DHA and DCC as a chemical coupling agent to afford the regiopure structured PC.

Preparation of CLA Ascorbyl Ester with Improved Volumetric Productivity by an Ionic Liquid-Based Reaction System. Zheng Guo[1], Bilian Chen[2,3], Bena-Marie Lue[1], Xuebing Xu[1,2], [1]Department of Molecular Biology, University of Aarhus, Aarhus, Denmark, [2]National Food Institute, Technical University of Denmark, Lyngby, Denmark, [3]Department of Biotechnology, Fujian Normal University, Fuzhou, Fujian, China

A new approach to the enzymatic production of conjugated linoleic acid (CLA) ascorbyl ester with aremarkably high volumetric productivity (120-200 g /L) has been developed, in which strong solvation by tOMA·TFA (methyltrioctylammonium trifluoroacetate) enables a high concentration of ascorbic acid to be applied, and in which t-butanol enhances conversion by changing the equilibrium constant of the activity coefficients. This work has experimentally demonstrated the practicability of achieving efficient reactions of polar compounds at high concentrations in ionic liquids without significant loss of enzyme activity.

Prevention and Alleviation of Metabolic Syndrome with Bioactive Phospholipids. Koji Nagao, Bungo Shirouchi, Nao Inoue, Masashi Inafuku, Teruyoshi Yanagita, Saga University, Honjo-1, Saga840-8502, Japan

The metabolic syndrome is a cluster of metabolic disorders that contribute to increased cardiovascular morbidity and mortality. Although the pathogenesis of metabolic syndrome is complicated, dietary lipids have been recognized as contributory factors in the development and the prevention of cardiovascular risk clustering. We investigated the physiological functions and molecular actions of bioactive phospholipids, such as omega3-PC and PI, in the development of metabolic syndrome. In the first study, omega3-PC diet significantly decreased omental WAT weight and markedly alleviated hepatomegaly and hepatic lipid accumulation in obese OLETF rats. In the second study, PI diet markedly alleviated hepatomegaly and hepatic lipid accumulation in obese, diabetic Zucker rats. Enzyme activities of hepatic injury markers in serum were significantly lowered in PI-fed Zucker rats. These results indicate that dietary bioactive phospholipids would be useful to prevent or alleviate metabolic syndrome in obese rats. In particular, the function of bioactive phospholipids as dietary adiponectin inducers deserves attention with respect to alleviation of metabolic syndrome by dietary manipulation. Reference: Prog Lipid Res, 47,127-146,2008; J Agric Food Chem, 56,2375-2379, 2008; J Agric Food Chem, 55,7170-7176,2007.

Effects of Structured Lipids Containing n-3 PUFA and Caprylic Acid on Lipid Profiles and Lymphatic Absorption of Rats. Junichi Nagata[1], Michio Kasai[2], Satoshi Negishi[2], Toshiaki Aoyama[2], Morio Saito[1,3], [1]National Institute of Health and Nutrition, Tokyo, Japan, [2]The Nisshin OilliO Group Ltd., Kanagawa, Japan, [3]Research Development Institute, MIKI Corporation, Hyougo, Japan

To examine the effects of some kinds of structured lipids (SLs) on lipid profiles and body fat accumulations in rats, we prepared the SLs containing caprylic acid (C8 or M) and EPA or DHA (n-3 PUFA or L) Male 4-wk-old Wistar rats were fed the experimental diets containing 3% (wt%) SLs and 1% (wt%) cholesterol for 4 wks. In addition, we

examined the lymphatic absorption of SLs from rat intestines. As a result, we observed that the serum cholesterol levels of rats fed SLs were significantly lower than those of the soybean oil diet. Serum and liver TG levels were significantly lower in rats fed EPA-C8-C8 type than those of the other groups. On the other hand, although there were no significant differences in the lymphatic absorption of n-3 PUFA and C8 from the intestines, MLM types might be absorbed more effectively than the LML types. These results indicate that the feeding of SLs containing medium-chain fatty acid and EPA could improve more effectively on serum and liver lipid profiles of rats.

AFTERNOON

LOQ 2 / PHO 2.1: Oxidation and Antioxidation of Neutral and Polar Lipids

Chair(s): J. Winkler-Moser, USDA, ARS, NCAUR, USA; and T. Wang, Iowa State University, USA

The Role of Plasmalogen Phospholipid in Transition Metal-induced Lipid Oxidation. G. Wang, T. Wang, Iowa State University, Ames, IA, USA

Plasmalogen is a glycerophospholipid that is found in animal tissues (heart, brain, and sperm) and bacteria. It contains a vinyl ether bond instead of an ester bond in the sn-1 position. It has been shown that both in vivo and in vitro plasmalogen contributes to the delayed or inhibited lipid oxidation, especially for polyunsaturated fatty acids in the presence of transition metal ions. This is assumed to be a direct interaction of transition metal with the enol ether double bond. In our study the interaction of plasmalogen and transition metal ions was investigated in an emulsion system. Our first aim was to evaluate how plasmalogen lipid oxidation happens in the absence/presence of each transition metal, e.g., copper and iron at different concentrations. Also, individual transition metal binding with enol ether bond will be evaluated under different pH levels (3 and 7). Our second aim was to investigate if different polyunsaturated fatty acids respond differently to plasmalogen in the presence of transition metal with respect to lipid oxidation as evaluated by peroxide value and thiobarbituric acid reactive substance. Our study offers insights on the antioxidant application of plasmalogen in food systems.

Investigations into Exploiting the Synergy of Lecithin with Natural-Source Antioxidants to Replace Synthetics. B. Sebree, Archer Daniels Midland Co., USA

Lecithin and its fractions have been studied for years as a synergist to primary natural sourced antioxidants to the inhibition of oxidation in oils. Olcott and Vander Veen (1963) evaluated lecithin fractions, while Brandt et al (1973)studied the pro- and antioxidant activity of phospholipids. Brandt ascribed antioxidant activity of phospholipids to 1) the regeneration of phenolic antioxidants and 2) complexation of pro-oxidant metals. The aim of this work was to attempt to exploit synergism of lecithin and well known chelators to enhance the activity of natural-sourced antioxidants to the point where they could be a viable alternative to synthetics. Primary antioxidants studied were mixed tocopherols, rosemary extract and green tea extract. A wide range of oil/fat systems were studied via the OSI-I instrument - tallow, lard, poultry fat, fish oil, DAG oil and a variety of vegetable oils. Success was very dependent upon the fat system being treated, but in most cases the use of lecithin in preparations enhanced antioxidant activity. Green tea extract was directionally more effective in most fat, as opposed to rosemary extract. Mixed tocopherols showed generally synergistic activity in conjunction with both green tea and rosemary extracts in a large number of the fat systems.

Oxidative Stability of Polyunsaturated Phospholipids. A. Takenaka, Y. Mizuno, M. Hosokawa, K. Miyashita, Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan

The nutritional importance of n-3 long chain highly-unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been proven beyond any doubt through research works across the globe. However, due to their high degree of unsaturation, EPA and DHA are much more susceptible to oxidation. On the other hand, they are relatively stable to oxidation in marine biological systems. In biological systems a large amount of EPA and DHA are present as phospholipids (PL) in the membrane. The present paper made clear the stability of HUFA as PL form with special reference to its synergistic activity tocoperol. EPA and DHA in PL were much more stable to oxidation under the presence of tocopherol than those in triacylglycerols. The mechanism responsible for the synergy of tocopherols and PL would be related to the involvement of amino group of PL in the enhancement of the

antioxidant activity of tocopherols and regeneration of tocopherols. Furthermore, when fish oil was oxidized with synthesized phosphatidylcholine (PC) and phosphatidylethanolamine (PE) having saturated and unsaturated fatty acids under the absence or presence of tocopherol, unsaturated PC and PE promoted fish oil oxidation without tocopherol. On the contrary, unsaturated PE showed synergistic antioxidant effect with tocopherol, while PC had little effect.

The Effect of Canola Oil Extracted from Pre-heated Seed on the Oxidative Stability of Oil-in-Water Emulsion Systems. A. Richards[1,2], P. Fagan[1,2], C. Ceccato[1,2], [1]CSIRO Food Futures National Research Flagship, Werribee, Victoria, Australia, [2]Food Science Australia, Werribee, Victoria, Australia

There is a push within the food industry to move towards ′clean labeling′ on food products. Therefore there is a need to identify and validate natural sources of antioxidants that provide equal or better protection towards oxidative deterioration than that currently used in the market place. The oxidative stability of canola oil can be improved through pre-heating of the seed. In this investigation, the effectiveness of canola oil extracted from pre-heated seed in stabilizing tuna oil-in-water emulsion systems against auto-oxidation was examined in comparison to (a) canola oil from non-treated seed, and (b) a selection of commonly used antioxidants. Oxidative deterioration is a significant problem within the food industry. EPA and DHA are both n-3 FA and the main component to undergo oxidation within tuna oil resulting in the formation of secondary oxidation products responsible for the fishy taste and smell. Results will be presented.

Antioxidative Activities of Mushroom (*Flammulina velutipes*) Extract Added to Bigeye Tuna Meat: Dose-dependent Efficacy and Comparison with Other Biological Antioxidants. Toshiaki Ohshima, Bao Huynh N. D. , Tokyo University of Marine Science and Technology, Tokyo, Japan

The ability of a hydrophilic extract prepared from edible mushroom (Flammulina velutipes) to stabilize fresh color of bigeye tuna (Thunnus obesus) meat was evaluated to compare it with certain other antioxidants. The fresh color shelf life of bigeye tuna meats, to which were added as 1, 3 or 5 mL of mushroom extract to 100 g of minced bigeye tuna meat, prolonged ice storage by more than 2, 4 and 6 days, respectively, in comparison with the control tuna meat without mushroom extract. Retarding oxidation of lipid in the tuna meat by the addition of 5 mL of mushroom extract to 100 g of minced bigeye tuna meat was more effective than adding ascorbic acid sodium salt (500 ppm) or α-tocopherol (500 ppm). The color changes significantly correlated with lipid oxidation as well as metmyoglobin formation in the tuna meat. These results clearly showed that the mushroom extract is a potential antioxidant which has ability to stabilize fresh color of tuna meat during ice storage.

Enhancement of Oxidative Stability of Canola Oil with Canola-derived Phenolic Antioxidants. C. Wijesundera[1,2], P. Fagan[1,2], A. Richards[1,2], C. Ceccato[1,2], [1]Food Science Australia, Werribee, Victoria, Australia, [2]CSIRO Food Futures National Research Flagship, Werribee, Victoria, Australia

Canola has the highest phenol content among commercial oilseeds. Ethanolic extracts of canola seed have been shown to possess strong antioxidant activity. During commercial canola oil extraction, where the seed is only heated to 75-100°C prior to oil extraction, very little of the canola phenolics is transferred to the oil. Heat treatment of the seed at higher temperatures (145-165°C) has been shown to increase the amount of phenolic compounds transferred to the oil, and also the oxidative stability of the crude oil. The increased oxidative stability has been attributed, at least in part, to 2,6-dimethoxy-4-vinylphenol (DMVP), which is derived from phenolic precursors occurring in canola seed. However, DMVP is almost completely removed during commercial oil refining. A practical way to utilize the antioxidant potential of DMVP is possibly to blend refined oil with relatively small amounts of crude oil extracted from high heat-treated canola seed. Experimental data on the relative oxidative stabilities of various blends will be presented.

PHO 2: Bioactive Lipids II
Chair(s): M. Ahmad, Jina Pharmaceuticals Inc., USA; and W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands

Ether Lipid and Plasmalogen Deficiency in the Mouse Central Nervous System. W.W. Just, University of Heidelberg, BZH, Heidelberg, Germany

Investigating the cerebellum of ether lipid (EL)- and plasmalogen (PL)-deficient mice, we observed: (i) defects in foliation patterning and delay in precursor granule cell migration, (ii) defects in myelination and concomitant reduction in the level of myelin basic protein, (iii) disturbances in paranode organization by extending the Caspr distribution and disrupting axo-glial septate-like junctions, (iv) impaired innervation of Purkinje cells (PCs) by both parallel fibers and climbing fibers (v) and formation of axon swellings by the accumulation of IP3 receptor-containing smooth ER tubuli. Functionally, conduction velocity of myelinated axons was significantly reduced. As PC innervation strongly depends on synaptic activity, presynaptic neurotransmission (glutamate and acetylcholine) was determined in isolated nerve terminals and found to be severely impaired. Assays of TBARS, glutathion and TRAP demonstrated that PLs are particularly sensitive to oxidative attack. In summary, these data show that EL deficiency results in severe developmental and lasting structural alterations at the cellular and network level of the cerebellum. Common molecular mechanisms that may underlie these phenotypes include organization and function of lipid raft microdomains and maintenance of PC Ca2+ homeostasis.

Polyelectrolyte and Cationic Peptides as Additives for Lung Surfactant Extracts. Edgar Acosta, Zdenka Policova, Michael Hair, Wilhelm Neumann, University of Toronto, Toronto, Ontario, Canada

Lung surfactants are mixtures of phospholipids and proteins secreted by the epithelial cells that line the alveoli of the lungs. Dipalmitoyl- phosphatidylcholine (DDPC) and dipalmitoyl-phosphatidylglycerol (DPPG) are two of the main saturated lipids present in lung surfactants. The role of these surfactants is to produce films at the liquid/air interface of the alveolar fluid and reduce the surface tension to near zero values upon compression (exhalation). This near zero tension guarantees that smaller alveoli don?t collapse due to Laplace pressure differences. While DPPC provides good mechanical stability to these surfactant films, DPPG molecules facilitate their formation, but also affect their stability. In this presentation we discuss the use of cationic additives to bind to the negatively charge lipids and offset some of the hydration effects. It will be shown that cationic peptides also have a profound effect on the mechanical properties of layer produces with mixtures of anionic (e.g DPPG) and zwitterionic (e.g. DPPC) lipids. The potential use of combinations of lung surfactant extracts and cationic peptides in new surfactant therapies for the treatment of respiratory distress syndrome (RDS) are discussed.

TUESDAY

AFTERNOON
PHO 3: Lecithin (Yesterday, Today, and Tomorrow)
Chair(s): W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands; and

History of Lecithin in the USA. G.R. List, USDA (Retired), USA

In the beginning, lecithin gums that separated in giant tanks as sludge became a financial opportunity in the 1930's.Many patents were issued in the 1940's,1950's and 1960's for the modification of lecithin to improve functionality with several modification that included halogenation, sulfonation, hydrogenation and hydroxylation. Applications were many.The 1970's and 80's were dedicated to technical support of the many potential applications developed for lecithin. Glidden/ Durkee had the largest research group dedicated to lecithin applications. In the 1990's the International Lecithin Study Group, ILSG became established as a not-for-profit organization registered as a New Jersey corporation as the International Lecithin and Phospholipid Society, ILPS. Later that decade the ILPS applied to the AOCS for the chartered Phospholipid Division. The turn of the century brought on interesting challenges like GMO and non GMO products in the 2000's. Organic products are becoming a growing market with the food sector.International lecithin supplies will continue to be a challenge for the US lecithin markets but with the ILPS and the AOCS Phospholipid Division the opportunities for all will expand into the next 100 years.

Phospholipids - Past, Present, Future - Alchemy to Mass Spectrometry. W. Shaw, Avanti Polar Lipids, Inc., Alabaster, AL, USA

The chemical knowledge of phospholipids and lipids that we currently possess is the product of the blood, sweat, tears, hard work, serendipity, and luck of the many giants that toiled in the past. We are all aware of the names of Chevreul, Thudichum, Bloor, Langmuir, Gorter, Pangborn, Folch, Bligh, Dyer, Carter, Hanahen, and Svennerholm. This list is by no means complete, but you get the idea that we are indebted to a great number of researchers. By some quirks of history another group of researchers existed that are not as well known. Among the less recognized giants are the alchemists Brandt, Godfrey and Boyle as well as the chemists Hensing, Franklin, Pockels, and Rayleigh. I will bring to life some of the fascinating research from this group.Phospholipids are major components of all biological cell membranes, and the development of membrane models that provide the basis for our current understanding of how membranes function will be reviewed. The importance of the membrane not only as the structural component of the cell but also as a repository of lipid molecules that generate lipid messengers and lipid second messengers will be highlighted. Modification of membrane phospholipids is the basis of this second messenger activity. Phosphatidylcholine, phosphatidic acid, phosphatidylinositides, and sphingomyelin are important molecules that contribute to the generation of second messengers. A brief discussion of cellular lipid transporters will be included.Analysis of phospholipids by several mass spectrometry techniques will point out the ease and power of the technology to define molecular species in biological samples. Two of the major questions in phospholipid research are, ?Why are there so many phospholipid molecular species (20,000)?? and, ?What do they do?? Current mass spectrometry techniques have the ability to identify phospholipid molecular species in tissue extracts and, unbelievably, to show the location of molecular species in tissue slices. In the future it will be possible to determine the location of lipid molecular species in individual cells and cell organelles. Will these data lead us to function? It is clear that we are just beginning to understand the importance of phospholipids and lipids in biological systems. The future looks bright and very exciting.

Lipid Analysis by NMR Using Cryo Probe Spectrometer Technology. Bernd W.K. Diehl, Spectral Service, Cologne, Germany

A new dimension of quantitative analysis of all lipid classesLipids mostly are complex mixtures of different organic molecules of natural sources. Triglyceride show different fatty acid compositions in the glycerol backbone at SN/1, SN/2 and SN/3 positions. In addition mono-and diglyceride as well as phospholipids and glycolipids can be detected and quantified. Other secondary plant ingredients like sterols can be analysed simultaneously. The NMR is a non destructive and absolute quantitative method. In former times the low sensitivity of NMR spectroscopy was a handicap of the method. Now the new cryo probe technology enhances the signal to noise ration by a factor of 5. A simultaneous increase of the magnetic field strength from 300 to 600 MHz results in an additional factor of 2.8. These combination of high field strength and cryo probe technique first time enables the use of 13C-NMR - the NMR active isotope with a low natural abundance of 1.2 % - for quantitative analysis of lipids within short measuring times, now comparable with a chromatography run. By the way, the well established 31P-NMR for all lecithin analysis, the I.L.P.S. reference method, has done a jump in sensitivity and dispersion, too.Several examples of quantitative NMR assays by 1H-NMR, 13C-NMR and 31P-NMR will be shown.

Improved Oil Recovery in an Adapted Enzymatic Degumming Process. W.D. Cowan[1], H.S. Yee[2], H.C. Holm[3], N. Korsholm[3], [1]Novozymes UK, Chesham, Bucks, UK, [2]Novozymes MY, Kuala Lumpur, Malaysia, [3]Novozymes A/S, Bagsvaerd, Denmark

Degumming of oils with phospholipase is the route demonstrating the lowest oil loss. This paper reports research into a shortened degumming process that aims to reduce the reaction time whilst maintaing the the high oil yields seen with standard process. Different phospholipases have been studied and data on the residual oil in gums and practical application of the technologies will be presented

History and Future of Lecithin in Europe. W. van Nieuwenhuyzen, Lecipro Consulting, Limmen, The Netherlands

Two companies made use of research on soy phospholipids modification:- (Nattermann) Phospholipid GmbH, Cologne with Phosphatidylcholine fractions made by chromatographic column separation for pharmaceutical and cosmetic uses. - Unilever with R&D on lecithins for anti-spattering agents in low salted margarines for kitchen use. Patented results were: -Alcoholic fractionation, -Enzymatic hydrolysis and -Acetone fractionation of acetylated lecithin. First 2

processes were started on plant scale in the 1960s.Entrepreneurial marketing was undertaken by Lucas Meyer CY, Hamburg with a range of lecithins with emulsifying functions for foods and for health supplements. This company was a breeding ground for staff members to start their own lecithin company. The applications and markets for fractions, hydrolyzed and chemical modified lecithins extended world wide. Modified lecithins from rape seed and sunflower source are now available. The drive is the recent IP non-GMO requirements. Business changes in oil mill ownerships and lecithin manufacturing responsibilities in the last decades are presented.Thanks to new analytical methods of Phospholipid composition and the use of novel (phospho) lipases, enzymatic esterification of phospholipids with specific healthy fatty acids is now possible. Other promising lecithins are dairy sphingolipids, marine phospholipids and phosphatidylserine.

WEDNESDAY

MORNING

PCP 4 / PHO 4: Protein Allergenicity and Regulatory Update on Phospholipids

Chair(s): J. Boye, Agriculture & Agri-Food Canada, Canada; J. Wanasundara, Agriculture & Agri-Food Canada, Canada; and W. van Nieuwenhuyzen, Lecipro Consulting, The Netherlands

Food Allergens and the Food Industry: Regulatory and Industry Perspectives Three Years After FALCPA. S. Taylor, University of Nebraska, Lincoln, NE, USA

The Food Allergen Labeling & Consumer Protection Act (FALCPA) in the U.S. and similar initiatives in other parts of the world have altered labeling practices and focused industry allergen control initiatives. In the U.S., FALCPA defined the commonly allergenic foods as milk, egg, fish, crustacean shellfish, peanuts, soybeans, tree nuts, and wheat. The common allergen lists differ in other countries. FALCPA mandates that the sources of ingredients derived from common allergenic foods shall be identied on the label in plain English language. This has been a major positive outcome for allergic consumers because it is now clear that casein is from milk and semolina is from wheat for example. However, FALCPA mandates labeling of allergenic sources on ingredients that may have quite low allergenic risks or that are present at rather low levels. Examples might include soy lecithin and fish gelatin. Exemptions from these labeling requirements are possible in the USA by petition but thus far no petitions have been granted. A much more workable petition process exists in the EU which has lead to the exemption of certain ingredients from labeling. Advisory or precautionary labeling (e.g. "may contain") has proliferated even though none of the regulations or statutes mandate its use.

Lecithin Regulatory Update Including Residual Proteins. W. van Nieuwenhuyzen, Lecipro Consulting, Limmen, Netherlands

Food processors need additives for producing foods with specific functions and stable shelf life. In USA Lecithins have CFR and GRAS status and comply with FCC regulation. The EU permitted food grade additive Lecithin, including fractions and enzymatic hydrolyzed lecithin is classified as E322.Following recommendations of the Codex Alimentarius the Regulatory Authorities world wide require the labeling of foods (additives) derived from foods with allergenic substances for information of the consumer. Lecithin Manufacturing Associations (ILPS, ELMA) have investigated in collaboration with TNO Food Research Institute and other Institutes that most commercial soy lecithins may contain residual soy proteins. Results of Elisa tests will be presented. Alcohol soluble lecithin fractions may be free of residual protein.As a consequence, the sourced origins such as "soy", ?egg? or ?dairy? lecithin have to be declared on the food label. European food processors require ingredients for non-GMO crops, even if the GMO is officially accepted by regulation. Identity Preservation schemes for sourcing raw materials are in force.

Impact of Thermal Processing on the ELISA Detection of Food Allergens. T.-J. Fu, U.S. Food and Drug Administration, Summit-Argo, IL, USA

Commercial ELISA test kits are increasingly used by food manufacturers to validate allergen control measures. Many of these tests are designed to allow quantitative analyses where the presence of target proteins is detected by binding

with specific antibodies. The concentration of the antigens is then determined from a standard curve generated with reference standards. Because quantitation is achieved via measurements of protein antigenicity, any changes in the antigenic property of the target protein may influence assay results. Thermal processing often leads to changes in the solubility and immunoreactivity of proteins. How thermal processing affects allergen quantitation by ELISA test kits remains to be determined. While many test kits employ antibodies that are raised against extracts of whole foods and are thus reactive to all protein components in these foods, other kits use antibodies raised against individual allergens and are therefore specific towards these proteins. Little is known about which tests are more suitable for detection of allergens in thermally treated food. This presentation discusses the impact of thermal processing on the performance of commercial ELISA kits for quantitation of egg, milk and peanut allergens. Factors such as the specificity of the antibody used, the thermal resistance of target proteins, and processing conditions used will be discussed.

Tree Nut Allergens. G.M. Sharma and S.K. Sathe, Florida State University, USA

Matrix Effects on Peanut Allergen Detection and Protein Extractability. J. Boye, N. Raymond, Agriculture and Agri-Food Canada, St-Hyacinthe, Quebec, Canada.

To assist in the detection of undeclared allergens in foods, reference materials with specified concentrations of targeted allergens are needed for the development and validation of allergen detection methods. In this study, protocols were developed for the preparation of peanut reference materials containing between 5 to 250 mg/kg of peanut using a peanut- and milk-free chocolate matrix. Specified amounts of peanut butter, defatted peanut flour and peanut protein isolate were added to pre-melted chocolate to give final concentrations in the desired range. The peanut extracts used were characterized using electrophoresis and RP-HPLC. Veratox and Tepnel Biosystems ELISA kits with and without extraction aids were used to measure the recoveries of the peanut proteins in phosphate buffer and the chocolate matrix. The results showed very low peanut recoveries in the chocolate matrices when no extracting aids were used. Recoveries improved with the use of extracting aids. Since sensitive peanut allergic patients can react to very low concentrations of peanut proteins, the research highlights the need for the use of extraction aids when detecting the presence of peanuts, especially, in chocolate matrices.

Identification and Characterization of Novel Soybean Allergens and the Creation of Hypoallergenic Lines. S. Gleddie[1], C. Gagnon[1], V. Poysa[2], E. Cober[1], [1]Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada, [2]Agriculture and Agri-Food Canada, Harrow, Ontario, Canada

Food allergies affect an estimated 2-5% of the North American population. Soy allergy is an emerging concern because of its widespread use in the food and beverage industry, and it currently ranks high among foods provoking allergic reactions among North American and Asian consumers. Soybean meal also provokes significant adverse IgE-mediated allergic reactions when fed to weaning piglets. We are currently identifying and characterizing the major soy allergens by 2-D immunoblotting and mass spectrometry using sera from North American soy sensitive patients. We will describe the major soybean allergens including several novel allergens. A suprising finding of this study is that some patients who suffer allergic reactions to specific soybean proteins do not cross-react to very homologous proteins. Epitope mapping is proceeding to further define the regions or domains responsible in some allergens. We have also developed high throughput methods to screen germplasm for lines which lack allergens, an approach which we hope will lead to 'non-GMO' lines which are hypoallergenic for the food industry.

Impact of High Pressure and Thermal Processing on the Immunoreactivity of Sesame Protein Isolate. Allaoua Achouri[1], Joyce Irene Boye[1], Vincent Nail[2], Lamia L'Hocine[1], [1]Agriculture & Agri-Food Canada, St-Hyacinthe, Qc, Canada, [2]Université de Reims Champagne, Ardenne, Reims, France.

Sesame seeds are used for the preparation of a range of traditional dishes and as decoration and flavouring agents by the bakery industry. The increasing consumption of sesame-containing foods, and the fact that sesame may be present as a hidden allergen, is likely to make sesame allergy become even more common in future. Sesame seed has been added to the list of food allergens in European countries and Canada but not in the United States. Although several

studies reported the existence of multiple allergens in sesame seeds, discrepancies persist in the literature due to factors such as the quality of extracts (purity), extraction conditions, food matrix and analytical techniques. Additionally, during food processing, proteins are subjected to various conditions, which lead to important conformational and structural changes that could be either beneficial or detrimental in terms of the immunoreactivity and/or allergenicity of the processed food. In the present study the extractability of sesame proteins under various conditions, their properties and the effects of high pressure (100-500 MPa) and thermal processing (boiling, dry roasting, microwave heating) on their immunoreactivity were investigated. Results obtained from these studies will be presented.

Recent Advances in the Development of Egg Allergy Immunotherapy. Y. Mine, M. Yang, University of Guelph, Guelph, Ontario, Canada

Increased awareness of food allergy and lack of efficient treatment regimens led to numerous investigations toward novel immunotherapeutic approaches. Recent epidemiological studies revealed that egg allergy prevails as one of the most common food hypersensitivities in industrialized countries. Egg allergens therefore constitute good models for the exploration of novel immunotherapies.Oral desensitization protocols have been assessed in patient cohorts with evidence of efficacy, but the approach remains associated with high risks of adverse effects and is unrealistic for highly-sensitive individuals. Safer and more efficient strategies are warranted. Identification and molecular characterization of egg components and their allergenic properties allowed the recent mapping of their T-cell and B-cell epitopes. These data have significantly facilitated exploration of elegant strategies, encompassing the use of non-IgE-reactive recombinant molecules and short T cell epitope-containing peptides. Promising results were recently obtained with use of murine models of egg allergy leading to elucidation of potential mechanisms involving regulatory T cells and key molecules such as TGF-b and FOXp3. Altogether, these investigations are expected to contribute to the successful development of clinically effective preparations for egg allergic patients.

Allergenic Proteins of Brown/Oriental and Yellow Mustard Seeds. J. Wanasundara, Y. Shim, Agriculture & Agri-Food Canada, Saskatoon, SK, Canada

Proteins that trigger allergenic reactions in human have been reported for yellow (Sinapis alba) and brown/oriental (Brassica juncea) mustard and caused the mustard allergenicity warning in Europe. In North America, mustard is a common condiment/spice particularly flour of yellow mustard is used in different foods. 2S proteins Sin a 1 of S. alba and Bra j 1E of B. juncea have been identified as molecules that are recognized by sera of mustard-sensitive patients. Sin a 2, a 11S protein of yellow mustard (YM) has also been identified as immunoreactive. Two immuno-dominant regions in the large polypeptide chain have been identified for Sin a 1. The allergenic 2S mustad proteins have 8-Cys motif, multiple S-S bonds, high resistance to pepsin digestibility and high thermal stability similar to many allergenic 2S proteins. Using pAb raised against purified Sin a 1 and Bra j 1E, several mustard cultivars was assessed. The Sin a 1 content ranged from 0.75 to 2.29 mg/g meal and Bra j 1E content from 0.40 to 0.75 mg/g for yellow and brown/oriental mustards, respectively. Detected Sin a 1 level of YM flour was reduced due to temperature of heat treatment and the duration of the treatment with a maximum reduction of 80%. Heating of aqueous YM flour slurry >15 min at 100°C resulted in a same reduction of Sin a 1 level as heating dry flour at 125°C for 60 min.

Assessing and Managing Food Allergy Risks: An Industry Perspective. Rene W.R. Crevel, Unilever Safety & Environmental Assurance Centre, UK

Food allergy is now well recognized as a public health issue and in several regions of the world new regulations to protect allergic consumers reflect this. Even before this, many parts of the food industry, responding to the concerns of consumers, implemented systems to manage the risks arising from allergens. Much experience has now been gained with such systems and the general principles that govern good practice are reasonably well understood. However, it is not always clear whether the implementation of such systems has been accompanied by a concomittant decrease in the frequency of product recalls attributable to food allergens. This observation may indicate that allergen management systems are inadequate, but an alternative hypothesis is that they reflect a growing awareness of the issues and readiness to act. This presentation will briefly review the principles underlying allergen management, consider gaps in knowledge that limit the ability to assess the risk from allergens accurately and attempt to draw conclusions about the adequacy of current systems.

AFTERNOON
PHO 5: General Phospholipids
Chair(s): L. Colbert, Archer Daniels Midland Co., USA; and S. Baseeth, Archer Daniels Midland Co., USA

Soy-Phospholipid Based Agricultural Adjuvants. Shireen Baseeth, Bruce Sebree, Archer Daniels Midland, Decatur, IL, USA

An adjuvant is any additive used in conjunction with an agrichemical to increase biological activity and/or to modify various physical properties of a spray solution. Based on the low eco-toxicological profile lecithin is gaining lot of attention as an emulsifier in many areas of pesticides and adjuvants. Soy-oil derived components provide excellent crop safety and replaces petroleum-based components with farm-grown components.In order to address this need, we have recently developed and introduced to the marketplace a new patent-pending adjuvant product produced from eco-friendly ingredients.In this presentation we report the performance properties of this lecithin based agricultural spray adjuvant. The product's superior ability in all adjuvant functions uniquely positions it as an ideal tank mix partner. Superior efficacy is achieved by providing measurable results in several areas like droplet size and coverage, deposition and retention, acidification and penetration, wetting/spreading and drift control. This product shows excellent synergy with commercial glyphosate formulations in both efficacy and retention. Field trials on soybean crop indicate no phytotoxicity and demonstrated excellent control and efficacy with glyphosate even on the most difficult to kill weeds.

^{31}P Nuclear Magnetic Resonance Phospholipid Profile of Soybean Emulsions Recovered from Aqueous Extraction. Linxing Yao, Stephanie Jung, Iowa State University, Center for Crops Utilization Research, USA

Aqueous extraction processing (AEP) is an environmentally friendly technology that extracts plant oils without using any organic solvent. The simultaneous extraction of other plant compounds, including protein and phospholipids (PLs), favors the formation of an oil-rich emulsion called cream, which traps the released oil. Optimization of AEP for maximum soybean oil extraction includes extrusion pretreatment and use of protease during aqueous extraction. The objectives of this study were to understand the role of PLs in stabilizing the emulsion and to identify how processing steps affect PLs profile. Determination of PLs profile was achieved with 31P NMR spectroscopy. PLs sample was prepared in a hydrated chloroform/methanol/Cs-EDTA solvent. The PLs profile in the emulsion recovered during AEP and enzyme-assisted AEP of full-fat soy flour, flake, and extruded flake were compared. There was evidence that PLs hydrolysis took place during extraction. The amounts of lysophosphatidylcholine and phosphatidic acid were increased after AEP regardless of starting materials. The extent of hydrolysis varied with starting materials. This study identified the parameters affecting the PLs profile and the emulsion stability and therefore provided strategies to control the cream emulsion properties.

Ultrasound-Assisted Extraction of Phospholipids from Palm-Pressed Fiber. S.C. Chua[1], C.P. Tan[1], O.M. Lai[2], K. Long[3], B.S. Baharin[1], [1]Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, [2]Faculty of Biotechnology and Biomolecular Sciences, Serdang, Selangor, Malaysia, [3]Department of Food Technology, Malaysia Agricultural Research and Development Institute, Serdang, Selangor, Malaysia

Extraction of phospholipids (PL) from palm-pressed fiber (PPF) using ultrasound technology was developed and quantified using SPE-HPLC-ELSD. PL that were considered in this work included phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC) and lyso-phosphatidylcholine (LPC). A central composite design (CCD) was employed to study the effect of ultrasound-assisted extraction (UAE) conditions namely amplitude (20%-90%), cycle (0.2-1.0 W/s) and sonication time (5-30 min) on the extraction yield of PL from PPF. The optimum parameters for the PL extraction were amplitude (20 %), cycle (0.2 W/s) and sonication time (30 min). Under the optimized condition, the response values obtained for overall extraction efficiency and individual extraction yield of PE and PC were 110 (mg/g), 12570 and 5426 (mg/kg), respectively. After UAE, ethanol was used to fractionate PC and PI. The sample to ethanol ratio also significantly ($p < 0.05$) affected the purity of PC in the PC-enriched fraction. The best ratio of sample/ethanol (g/mL) was 1:20 which gave the highest yield of PC fraction, with the highest level of PC in the PC fraction and the highest level of PI in the PI fraction. The PC-enriched fraction containing 85% PC, 6% PE

and 9% PI based on the total PL content, whereas the PI-enriched fraction contained 19% PC, 10% PE and 71% PI.

A New Food Grade Organogelator: Evolution of Lipid Structuring. Michael Rogers[1], Amanda Wright[2], Alex Marangoni[2], [1]University of Saskatchewan, Saskatoon, SK, Canada, [2]University of Guelph, Guelph, On, Canada

The quest for a simple food grade organogelator has been ongoing since the potential of these novel soft materials was recognized. Potential applications for organogels in the food industry are numerous, ranging from limiting oil migration in multi-component foods to replacing trans and saturated-fats in spreadable products to encapsulating nutraceuticals. In this work, we explore the use of ceramides, a class of polar lipids, to gel vegetable oil. Egg and milk sphingomyelin, enzymatically converted using phospholipase C to ceramides, can be incorporated into vegetable oil producing stable gels. The microstructure varies from "fiber-like" networks to "dendritic-like" crystal networks. This microscopic diversity allows for the fine-tuning of not only the microscopic properties of ceramide-based gels but also the physical properties.

Oil Yield Improvement by Enzymatic Water Degumming. J.B. Soe, Danisco, Brabrand, Denmark

BackgroundThe process of enzymatic degumming has so far not gained a strong foothold in the crushing and oil refining industry. This is probably because the implementation of the enzyme degumming process has previously required heavy investment in tanks and utilities This presentation focuses on a new enzyme - lipid-acyltransferase – for water degumming process with improved oil yield.Lipid-acyltransferase (or phosphatidylcholine sterol O-acyltransferase) is a new generation of enzymes for yield improvement in the water degumming process. A critical novel feature of lipid-acyltransferase is its ability to hydrolyse various phospholipids and transfer the released fatty acids to sterols. During water degumming of crude vegetable oil, the enzyme reacts on phospholipids such as lecithin, promoting the formation of lysophospholipids and sterol esters. Partial degradation of phospholipids also changes the consistency of the gum. Furthermore, the separation by continuous centrifugation reduces the amount of oil remaining in the gum phase. The enzymatic water degumming process is easily implemented – no capital investment, no change in plant configuration, no change in process time. Examples of enzymatic water degumming with lipid acyltransferase will be given, including chemical and physical analysis of oils and gums.

Effect of Chemical Environment on Soybean Protein-Lecithin Interaction and Stability of O/W Emulsions. J.R. Wagner[2], M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas (UNLP-CONICET), La Plata, Pcia. de Buenos Aires, Argentina, [2]Universidad Nacional de Quilmes, Bernal, Pcia. de Buenos Aires, Argentina

The aim of this work was to study the effect of the chemical environment on soybean protein-lecithin interaction and the stability of O/W emulsions. O/W (25:75 w/w) emulsions were prepared with native(NSI) or denatured (DSI) soy isolates, lecithin (Lec), sunflower oil, protein-lecithin ratio (100:1-10:1), pH 2-7.The effect of pH, ionic strength and calcium salts were studied. The optical characterization of dispersions were determined. For DSI-Lec and NSI-Lec systems, creaming rate changed as a function of pH value. For DSI-Lec the stability increased at pH values not closed to the isoelectric point. The presence of Lec enhances the stability against to the coalescence process. It is interesting to consider the effect of the unfolding of 7S and 11S fractions by thermal treatment and their increased surface hydrophobicity on soybean proteins-Lec interaction. NSI-Lec emulsions at pH 2.0 presented an important initial emulsifying activity by denaturation of 11S fraction but the creaming rate was faster than those obtained for pH 5.5,7.0. The increase of ionic strength diminished the interactive effect of soy protein-lecithin systems with an enhance in creaming rate by flocculation/ coalescence of droplets.The increase in calcium concentration showed changes in emulsion stability due to the destabilization of the interfacial film by charge screening of counterions.

Enzymatic Hydrolysis and Chemical Characterization of Sunflower Lecithins. D.M. Cabezas[1], R. Madoery[2], B.W.K. Diehl[3], M.C. Tomás[1], [1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)-Facultad de Ciencias Exactas (UNLP-CONICET), La Plata, Buenos Aires, Argentina, [2]Cátedra de Química Orgánica, Facultad de Ciencias Agrarias, UNC, Córdoba, Córdoba, Argentina, [3]Spectral Service GmbH, Laboratorium für Auftragsanalytik, Cologne, Germany

Lecithins are used in a wide range of industrial applications. Modification process such as hydrolysis can originate lysoderivates applied to different food purposes (emulsions, bakery products) due to the interesting functional properties of lysophospholipids (LPL). The aim of this work was to study the enzymatic hydrolysis of sunflower lecithins investigating the effect of different operative conditions on the phospholipid (PL) composition of modified lecithins. The enzymatic hydrolysis of sunflower lecithin was carried out in a lab reactor with a phospholipase A2 (Lecitase 10 L), $CaCl_2$ (0.1-0.4 M) (cofactor), initial pH values 7-9, 60°C, t (0.6-5 h) under continuous agitation. The reaction products were deoiled and dried under vacuum. The PL composition of the samples was determined by ^{31}P NMR. The results showed that the application of this modification process allowed to obtain sunflower lysolecithins with a total LPL content ranged from 21 to 37% in comparison with the starting sunflower lecithin (1%). The highest concentration of LPL was recorded for $CaCl_2$ 0.1 M, pH 7 and both times of incubation. The conversion of the main original PL was up to 85%, for all conditions assayed, in this order: PC > PI and PE. The sunflower lysolecithins constitute a potential and interesting alternative as bioemulsifiers to be applied at the food industry.

Antioxidant and Functional Properties of Novel Quercetin Enriched Lecithin. M.F. Ramadan, Zagazig University, Zagazig, Egypt

Antioxidative activities of native soy lecithin and mixtures of quercetin and lecithin (1:1, w/w) in the protection of triolein models stored under accelerated oxidative conditions for 15 day in the dark at 60 °C were studied. The progress of oxidation was followed by recording the ultraviolet absorptivity and measuring the formation of oxidative products (peroxide value). The antiradical action of different models against DPPH radicals was screened during Shaal oven test. The factors influencing the oxidative stability of different triolein models were also discussed. Inverse relationships were noted between peroxide values and oxidative stabilities at termination of the storage. Absorptivity at 232 nm and 270 nm in models containing lecithin increased gradually with the increase in time, due to the formation of conjugated dienes and polyenes. In general, oxidative stabilities of quercetin-lecithin enriched models were better than in models containing lecithin or quercetin alone, most likely as a consequence of synergism between polar lipids and quercetin. Moreover, increases in concentration of quercetin-lecithin mixture resulted in an increase in its antioxidative activity. These results may be useful for improving the antioxidative activity and health impact of commercial lecithin in different food applications.

Phospholipid Posters

Production of Structured Phospholipids by an Immobilized Phopholipase in a Batch Reaction System: Effect of Water Activity.

In-Hwan Kim[1], Hugo S. Garcia[2], Charles G. Hill[3], [1]Korea University, Seoul, 136-703, Republic of Korea, [2]UNIDA, Instituto Tecnol´ogico de Veracruz, Veracruz, Ver. 91897, Mexico, [3]University of Wisconsin-Madison, Madison, WI 53706, USA

Structured phosphatidylcholine (PC) was synthesized in a solvent-free system using an immobilized enzyme prepared from commercially available phospholipase A1, namely, Lecitase® Ultra, from *Thermomyces lanuginosus/Fusarium oxysporum*. PC derived from soybean and the fatty acids obtained by saponification of a fish oil were used as substrates for synthesis of structured PC by phospholipase A1-catalyzed acidolysis. The effects of water activity, and enzyme loading on incorporation of n-3 PUFA into PC were ascertained by monitoring the time course of these reactions. The water activity range tested in this study was between 0.11 and 0.95. The acidolysis reaction was accompanied by a parallel increase in the extent of hydrolysis, producing a significant decrease in the yield of PC. Consequently, when both incorporation of n-3 PUFA and the yield of PC are considered, two water activities (0.53, and 0.65) were selected as an appropriate compromise between these factors.

Phospholipase-Catalyzed Acidolysis of Phosphatidyl Choline with n-3 PUFA from Fish Oil.

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Phosphatidylcholine (PC) was successfully modified by phospholipase A1-catalyzed acidolysis with the fatty acids obtained by saponification of fish oil. The resulting phosphatidylcholine contains significant levels of eicosapentaenoic (EPA; C20:5), docosapentaenoic (DPA; C22:5), and docosahexaenoic (DHA; C22:6) acid residues. Modification of the PC was accomplished in a solvent-free system using phospholipase A1 from *Thermomyces lanuginosus/Fusarium oxysporum* as the biocatalyst. The effects of variations in the reaction parameters, namely, reaction time, enzyme loading, temperature, and vacuum on the time course of the reaction were investigated. After only 6 h of reaction at 55 ^{o}C, 21.0 mole % n-3 polyunsaturated fatty acids (PUFA) (sum of EPA, DPA, and DHA) was incorporated into PC at a loading of the enzyme solution of 10% of the total weight of substrates. As the reaction progressed, incorporation of n-3 PUFA reached a maximum of 28.0 mole % at 24 h. Reaction times longer than 6 h led to higher incorporation of n-3 PUFA but were associated with significant decreases in the yield of PC. Even though application of a vacuum produced a higher yield of PC, there was a parallel decrease in the extent of incorporation of n-3 PUFA into PC.