106th AOCS Annual Meeting and Industry Showcases

Health and Nutrition Division Technical Program Abstracts

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The presenter is the first author or otherwise indicated with an asterisk (*).

H&N 1: Lipids and Lipid Mediators Throughout the Lifespan

This session is sponsored in part by DSM and Johnson & Johnson

Chairs: C.J. Lammi-Keefe, Louisiana State University, USA; and A.P. Kitson, University of Toronto, Canada

Impact of Dietary n-3 PUFA Deficiency on Neuroimmune Interactions in the Developing Brain: Relevance for Behavioral Impairment. A. Nadjar, C. Madore, C. Bouju-Bosch, A. Thomazeau, C. Lacabanne, Q. Leyrolle, C. Joffre, and S. Layé*, NutriNeuro, France.

The corollaries of the obesity epidemic that plagues developed societies are malnutrition and resulting biochemical imbalances. Low levels of essential n-3 PUFAs have been linked to cognitive deficits, but the underlying mechanisms are mostly unknown. We hypothesized that a low dietary intake of n-3 PUFAs during development impairs microglia, the main innate immune system cell in the brain and, in turn leads to an inappropriate neuronal network and cognitive and emotional deficit later in life. Herein, we assessed the effect of a dietary deficiency in n-3 PUFAs during development on mice memory and anxiety using various behavioral tasks. In order to evaluate the integrity of the microglia activity and synaptic network in those animals, microglia phagocytic activity, spine density and electrophysiological activity were analyzed in the brain of offspring at post-natal days 21. Our results indicate that developmental n-3 PUFA deprivation leads to a selective decreased spatial memory at P21 and in adulthood. Microglia phagocytic activity was impaired at P21 but not at adulthood. In addition, synaptic plasticity and spine density were altered in the hippocampus. These findings identify microglia-neuronal interaction as a plausible mechanism underlying the memory alterations caused by the n-3 PUFAs deficiency during brain development.

Effect of Oxidized Oil Consumption on Biomarkers of Atherosclerosis in LDLr Knockout Mice. M.S. Nogueira, M.C. Kessuane, B. Cogliati, and I.A. Castro*, University of São Paulo, Brazil.

Our hypothesis is that chronic intake oxidized fatty acids in a high fat diet could promote atherosclerosis. To evaluate this hypothesis, C57BL/6 mice were separated into 5 groups. One group was composed by wild type animals and received diet containing 4% soybean oil (CONT-). The other four groups were composed by LDLr $^{-/-}$ animals and received a high fat diet containing 20% partially oxidized linseed oil + 10 % lard. Among these four groups under high fat diet, one group had type 1 diabetes induced by streptozotocin (CONT+), while in the other three groups, linseed oil was previously heated for 0h (LOW), 5h (MED) and 10h (HIGH). Hydroperoxide, TBARS and hexanal values of these three linseed oil samples were: 2.5, 3.8 and 4.7meq/L; 3.7, 6.3 and 11.7mg/L and 0.0, 29.6 and 37.4pg/mL respectively. After 90 days, WT mice under regular diet showed lower plasma concentrations of total cholesterol, HDL-c and LDL-c than LDLr $^{-/-}$ mice fed with high fat diet. CONT+ animals had lower body weight gain as consequence of diabetes induction. Among the mice fed with high fat diet, groups HIGH and CONT+ showed higher malondialdehyde values in the liver (1.24 and 1.35mmol/mg pt/mg PUFA) than groups LOW and MED (0.72 and 0.68mmol/mg pt/mg PUFA), suggesting that the chronic intake of oxidized oils could increase the oxidative stress in vivo.

A Possible Relation of Serum Fucoxanthinol Levels and Glucose Metabolism in Japanese Adults: Rumoi Fucoxanthin Intervention Study 1. N. Mikami[1], M. Hosokawa[2], M. Abe[2], K. Miyashita[2], H. Sohma[1], and Y. Kokai[1], [1]Sapporo Medical University School of Medicine, Japan, [2]Hokkaido University, Japan.

Although fucoxanthin (Fx) is a marine carotenoid found in brown algae and has been reported anti-obesity and anti-diabetic effects in vivo animal models, little is known about a relation between Fx bioavailability and its physiological effects in humans. In this study, we designed a randomized clinical intervention trial to investigate effects of continuous Fx intake in Japanese subjects. Sixty healthy adult participants with BMI more than 22 were randomly and blindly assigned to three groups. Participants were instructed to take capsules (containing 0, 1 and 2mg Fx for respective groups) every day for 8 weeks. Before and after the trial, we measured serum fucoxanthinol (Fx metabolite) levels through LC-MS/MS system we developed previously and biochemical parameters associated with life-style related diseases.

Serum fucoxanthinol levels in the Fx 1 and 2 mg groups increased significantly compared to the 0 mg group. In rates of change of biochemical parameters, there were no significant differences among the three groups on lipid metabolism. In contrast, the

rate of HbA1c was significantly lowered in the Fx 2 mg group compared to the 0 mg group.

Our results indicate a possible relation between serum fucoxanthinol levels and glucose metabolism after Fx intake in Japanese subjects.

GPS2 at the Crossroad of Lipid Metabolism and Inflammation in Mouse Adipose Tissue. C. Cederquist, C. Lentucci, H. Johnson, M. Cardamone, and V. Perissi*, Boston University, USA.

Obesity-induced defects in insulin signaling lead to the development of insulin resistance and Type II Diabetes (T2D). Currently, in the United States, the large majority of adults are overweight or obese, with roughly 60 millions of Americans being at a significantly higher risk for several diseases including diabetes and hypertension. G-protein pathway suppressor 2 (GPS2) has recently emerged as a novel and important regulator of homeostasis and inflammatory responses in metabolic organs. Previous work from our lab and others indicates that GPS2 is a multi-functional protein shuttling between the cytosol, where it inhibits activation of the stress kinase JNK as a modulator of ubiquitin signaling downstream of TNFalpha receptor, and the nucleus, where it regulates gene expression at the level of gene transcription by inhibiting pro-inflammatory gene targets and activating key mediators of the lipolysis pathway. Characterization of tissue-specific knock-out and overexpression mice will be presented to confirm the critical role played by GPS2 in the regulation of obesity-induced inflammation and lipid metabolism *in vivo*.

Examining Temporal Changes in Docosahexaenoic Acid Status During Pregnancy Using Lipidomics and Transcriptomics. A. Chalil[1], A.P. Kitson[1], J. Aristizabal Henao[1], K. Marks[1], J. Elzinga[1], F. Badoud[2], D. Mutch[2], and K. Stark*[1], [1]University of Waterloo, Canada, [2]University of Guelph, Canada.

Docosahexaenoic acid (DHA) increases in maternal plasma during pregnancy. Pregnant rats were fed a Total Western Diet based chow with (TWD+) or without (TWD-) DHA, or a standard rat chow low in DHA as an additional control. Rats (n=6 per group at each time point) were sacrificed before pregnancy, at 15 and 20 days of pregnancy, and 7 days postpartum. At day 20, DHA concentration in plasma lipids of all diets was increased (3-6 fold) compared with other time points. A similar, but less dramatic response (1.5 fold) was observed in liver. Lipidomic analyses indicated that 16:0/22:6n-3 phosphatidylcholine accounted for most of the increase at day 20. Protein and mRNA assessment indicated that Δ6-desaturase, elongase 5 and phosphatidylethanolamine methyltransferase were upregulated slightly at day 20. Transcriptomic analysis suggested that glycerophospholipid synthesis was shifted away from phosphatidylethanolamine and towards phosphatidylcholine but there was no evidence of specific gene products responsible for the increase in 16:0/22:6n-3 phosphatidylcholine. The increase in DHA during pregnancy appears to be under complex control that likely involves fatty acyl synthesis, transport and incorporation, and the molecular players involved may be regulated at the level of protein abundance and/or activity rather than mRNA expression.

Maintaining Brain PUFA Concentrations: Uptake Mechanisms and Rapid Metabolism. R.P. Bazinet, University of Toronto, Canada.

The brain is especially enriched with the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA) and arachidonic acid, while being virtually devoid of other PUFA such as eicosapentaenoic acid (EPA). It has been suggested that the plasma supply to the brain regulates brain PUFA levels. Candidate plasma pools that supply the brain with PUFA include the plasma unesterified pool, PUFA esterified to lysophosphatidylcholine or the uptake of PUFA-containing lipoproteins via lipoprotein receptors into endothelial cells of the blood brain barrier. This paper will present recent studies that have examined the role of lipoprotein receptors and the kinetics of candidate plasma pools which supply the brain. Upon presenting evidence that the plasma unesterified pool is a major source of brain PUFA, especially for DHA, I will describe how rapid metabolism also maintains very low levels of certain PUFA, such as EPA. Because fatty acid uptake into the brain can be imaged, we can estimate brain PUFA, including DHA, requirements. A better understanding of how PUFA enter the brain could lead to new therapeutics to target as well as new insights into brain function in health and disease with fatty acid imagining.

H&N 2: Evaluating Lipids in Human Trials

This session is sponsored in part by PepsiCo

Chairs: M.A. Belury, Ohio State University, USA; and S. Raatz, USDA, ARS, USA

Planning Clinical Trials with Oils. P.J.H. Jones, University of Manitoba, Canada.

Evaluating the health benefits of oils and oil-based bioactives requires nutrition-based clinical trials. However, the degree of certainty of this evaluation is dependent on the quality and reproducibility of such clinical trial data. Robust communication of food and nutrition science is needed to facilitate the adoption of healthful dietary practices among the consumer public for the proactive prevention of chronic disease; thus, the design and implementation of these randomized clinical trials (RCT) must be of the highest quality and integrity. Currently, limited resources are available for defining the various steps involved in an oil-based RCT establishing a need for a comprehensive guidance document outlining the best practices for the design, conduct and report RCTs to promote the fastidious generation and dissemination of quality clinical trial data. Accordingly, this presentation defines the various steps of a oil-based human clinical trial from inception to publication from a food health perspective.

Conducting Randomized Clinical Trials with Dietary Oils: Issues for Design and Interpretation. M.A. Belury, Ohio State University, USA.

Clinical trials seeking to elucidate novel role of lipid-soluble components on the diet are fraught with ambivalent outcomes. Reasons for the ambivalence could be attributed to the belief that double-blinded randomized clinical trials are the only gold standard for clinical proof of a biological effect. In addition, there has been a negligence in many reports of addressing purity and quantity of compounds, and accuracy of describing other aspects of dietary materials to be tested (e.g., active, or “test”, components and inactive, or “comparative,” components). These issues raise the question: Do clinical researchers need to re-think how clinical trials with dietary oils are designed and interpreted before confidence is lost for clinical lipodology? A few examples from the literature will be reviewed of studies where critical details have helped close the gap for improving the study and new knowledge of dietary lipids in human health. As a conclusion, a brief will list criteria that could improve the rigor and impact of clinical studies of dietary oils.

Total Dietary Fat and n-3 Fatty Acids Intake Modify Plasma Phospholipid Fatty Acids, Desaturase Activity Indices, and Urinary Prostaglandin E. S. Raatz[1,2], [1]USDA, ARS, USA, [2]University of Minnesota, USA.

Compared to diets high in fat, low fat diets are associated with reduced risk of cardiovascular disease. We hypothesized that a low fat (LF; 20% fat) and a low fat high omega-3 (n-3) fatty acid diet (LFn3; 23% fat with 3% as ALA, EPA and DHA) would enhance n-3 composition of PLFA and reduce urinary prostaglandin E2 (PGE2) relative to a high fat diet (HF, 40% fat) and that these changes would be associated with alterations in delta 5 and 6 desaturase (D5D, D6D) activity. Phospholipid fatty acids and urinary PGE2 were measured and D5D and D6D activity indices calculated in a cross-over trial in 17 postmenopausal women fed each of three test diets (HF, LF, LFn3) for 8-week feeding periods. Desaturase activity indices were calculated as D5D: 20:4n-6/20:3n-6 and D6D: 20:3n-6/18:2n-6. PLFA ALA, EPA, DPA, DHA and total n-3 fatty acids increased while LA and ARA decreased with consumption of LFn3. The LF resulted in enhanced ARA and DHA. HF reduced D6D while both HF and LF increased D5D. Urinary PGE2 was reduced in response to both the LF and LFn3 diets.

Testing a Mixture of Fats and Oils for Use as a Placebo in Clinical Trials. T. Orchard, M.A. Belury, R. Cole, R. Andridge, X. Pan, J. Lester, A. Logan, L. Yee, and M. Lustberg, Ohio State University, USA.

Objective: Determine safety and stability of mixed fats and oils in a placebo for use in randomized controlled trials.

Methods: Softgel capsules were manufactured containing placebo formulation based on the ratio of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the typical American diet; appearance matched treatment capsules (fish oil). Quality testing was performed yearly, in triplicate, using gas chromatography during study enrollment (ending 2.5 years after placebo manufacturing). Safety was assessed by adverse event reporting and serum lipids after 24 week

supplementation period in 35 breast cancer survivors.

Results: Mean SFA, MUFA, PUFA (omega-6 and omega-3) content of placebo capsules at baseline was 33.1%, 38.5%, 28.2% (26.5% and 1.6%), respectively. Measured values were within 0.5-8.2% of values predicted by formulation. Composition remained stable over 2.5 years for all categories except MUFA, which increased 0.9% (p=0.01) in year 1. No major adverse events were reported in placebo group. Groups did not differ in incidence of hypercholesterolemia [placebo: (1/17(6%), treatment: 3/18(17%), p=0.60] or hypertriglyceridemia [placebo: 5/17(29%), treatment: 1/18(6%), p= 0.09].

Conclusions: A softgel placebo similar in fatty acid ratio to the typical American diet was stable for 2.5 years and was safe and well-tolerated in breast cancer survivors.

Understanding Patterns of Incorporation of Fatty Acids in Humans. P.C. Calder, University of Southampton, UK.

In the blood, fatty acids are found as esterified components of complex lipids (e.g. TG, PL) within lipoproteins or in non-esterified form. Cell membranes contain fatty acids as esterified components of complex lipids, mainly PL. There are several types of PL in lipoproteins and cell membranes. Fatty acids are stored as TG in adipose tissue. The fatty acid composition of the various transport (lipoprotein, NEFA), storage (adipose tissue TG) and functional (cell membrane PL) pools of fatty acids is different – each lipid, cell and tissue type has a characteristic composition. However, it is possible to alter fatty acid composition through changing intake of certain fatty acids. Human studies with alpha-linolenic, stearidonic, gamma-linolenic, conjugated linoleic and arachidonic acids will be summarised focusing on plasma PL and blood immune cells. The incorporation patterns of the omega-3 fatty acids EPA and DHA have been most extensively evaluated. In most cells and tissues the content of EPA and DHA is low compared with the content of omega-6 fatty acids. Increasing EPA and DHA intake increases the EPA and DHA content of blood lipids, blood cells, and many tissues – the effect is dose, time and tissue dependent with all pools eventually reaching a new steady state. Rapidly turning over pools (e.g. plasma PL) change fatty acid composition more quickly than slowly turning over pools. Because the relationship between EPA and DHA intake and “status” is linear, many sites can be used to monitor omega-3 fatty acid intake. Plasma lipids, especially PL, and red blood cells are commonly used. The latter represent fairly long term intake while the former can represent short term changes. Other blood lipids and other blood cells types (platelets, immune cells) also provide a good reflection of omega-3 intake. Factors known to affect omega-3 fatty acid incorporation will be highlighted.

H&N 3: Classes of Saturated Fatty Acids and Health Implications

This session is sponsored in part by Johnson & Johnson, Nestlé S.A., and The Beef Checkoff

Chairs: F. Dionisi, Nestlé, Switzerland; and E.A. Decker, University of Massachusetts Amherst, USA

Individual Saturated Fatty Acids and Risk of Cardiovascular Disease: Epidemiologic Evidence. F. Hu, Harvard T.H. Chan School of Public Health, USA.

Types of dietary fat have been shown to have distinct relationships with risk of cardiovascular disease. Compared to polyunsaturated fatty acids (PUFAs), dietary saturated fatty acids (SFAs) increase total and low-density lipoprotein (LDL) cholesterol and are associated with elevated risk of developing coronary heart disease (CHD). Increasing evidence suggest that different SFAs may have different effects on CHD risk. In the Nurses' Health Study cohort, we reported that dietary intake of short- to medium-chain SFAs (4:0–10:0) was not associated with CHD risk, while longer-chain SFAs (12:0–18:0) were each associated with a non-significantly increased risk of CHD in multivariate analyses adjusted for cardiovascular risk factors. Substitution of longer-chain saturated fatty acids with PUFAs is associated with significantly lower risk of CHD. In addition, we examined circulating very-long chain saturated fatty acids (VLCSFAs (C20:0, C22:0, and C24:0) in plasma and erythrocytes and risk of CHD and found that plasma VLCSFAs were independently associated with favorable profiles of blood lipids and other CVD risk markers such as fasting insulin and C-peptide. Higher concentrations of VLCSFAs were also associated with a significantly lower risk of CHD. The mechanisms underlying these observations are not well established, although evidence from animal experiments suggests that these fatty acids may actively modulate lipid metabolism and insulin sensitivity through multiple pathways.

Dairy Foods, Dairy Fat, and Cardiometabolic Outcomes. D. Mozaffarian, Friedman School of Nutrition Science and Policy, Tufts University, USA.

This talk reviews the current evidence, knowledge gaps, implications, and future directions for dairy fat and cardiometabolic health. The role of dairy products, and especially dairy fat, in cardiovascular and metabolic health is controversial. Some epidemiological studies have suggested beneficial effects of low-fat dairy consumption on hypertension and stroke risk, and low-fat dairy consumption is a component of the beneficial Dietary Approaches to Stop Hypertension (DASH) diet. Conversely, consumption of whole-fat dairy has been discouraged, largely due to concern for potential adverse effects of saturated fat on coronary heart disease as well as due to higher calories in whole-fat products. However, accumulating evidence suggests that dairy fat is not harmful, and may indeed be beneficial, for cardiometabolic health, in particular type 2 diabetes. Certain types of dairy foods, such as yogurt, may also be beneficial for weight gain. This growing evidence suggests that dairy fat might reduce insulin resistance and type 2 diabetes, with benefits greatest for cheese and yoghurt. Further research is needed to establish whether specific fatty acids components, such as odd-chain saturated fats, median-chain saturated fats, or specific natural ruminant trans fats, may have direct physiological benefits or are merely correlates of other beneficial compounds in dairy fat or other aspects of dairy fat-rich foods, such as probiotics or fermentation. Whatever the mechanism, these data add further challenges to prevailing dietary guidelines that recommend low-fat dairy products on the basis of the postulated bone benefits of calcium and theorized harms of total saturated fat and calorie content, rather than consideration of the complex nutrients and preparation methods of different dairy foods and the evidence for their direct health effects.

Reevaluating Effects of Dietary Saturated Fats on Human Health. Fatty Acid Profile or Food Factors Induced by Processing? J.T. Brenna and K.S.D. Kothapalli, Cornell University, USA.

Saturated fats, and their cousins *trans* fats, are the general names nutritionists apply to solid fats. Human nutrition studies almost invariably ascribe major effects of all fats to a featured component that may, or may not, be the predominant chemical entity, e.g. saturated fatty acids (FA) and *trans* (FA), whether or not supported by animal studies on that component. Saturated fats are complex lipid mixtures that should not be confused with specific FA because components <1% can dominate biological effects. Animal studies show that the inherently saturated coconut oil's effects on circulating lipids are independent of FA profile and depend on processing method, whether "virgin" or RDB. Cocoa butter, a highly saturated tropical fat

seldom harshly processed, is regarded as cardioprotective. Positive effects of these saturated fats are often ascribed to their content of lauric acid and stearic acid, respectively. Recent meta-analyses of saturated fats conducted without regard to the macronutrients for which they substitute (oils or carbs) show no significant atherogenicity. In contrast, the AHA asserts convincing evidence that substitution of unspecified PUFA for saturated fat reduces atherogenicity, a point currently disputed. Too little attention has been focused on the acquisition of atherogenic properties during fat processing.

Health Effects of Dietary Stearic Acid Compared with Other Saturated, Unsaturated, and *trans* Fatty Acids. J.E. Hunter[1], J. Zhang[2], and P.M. Kris-Etherton[2], [1]Xavier University, USA, [2]Pennsylvania State University, USA.

This presentation covers clinical studies we reviewed that evaluated the relation between dietary stearic acid (STA) and cardiovascular disease (CVD) risk factors, including plasma lipids and lipoproteins, hemostatic variables, and inflammatory markers. Compared with other saturated fatty acids, STA lowered LDL cholesterol, was neutral with respect to HDL cholesterol, and directionally lowered the ratio of total to HDL cholesterol. Considering unsaturated fatty acids, STA tended to raise LDL-C, lower HDL-C, and increase the ratio of total to HDL cholesterol. Three studies showed increased plasma fibrinogen when dietary STA exceeded 9% of energy. (The current 90th percentile of intake is 3.5% of energy.) Two studies showed no significant change in the inflammatory marker C-reactive protein after feeding high-STA diets compared to diets high in either oleic acid or myristic and lauric acids. One-to-one substitution of STA for *trans* fatty acids (TFAs) showed a decrease or no effect on LDL-cholesterol, an increase or no effect on HDL-cholesterol, and a decrease in the ratio of total to HDL cholesterol. Research is needed to evaluate effects of STA on emerging CVD risk markers, such as fibrinogen, and to understand the responses in different populations.

Saturated Fatty Acids and Inflammation. P.C. Calder, University of Southampton, UK.

Inflammation underlies many common conditions. Dietary factors can be both a trigger and a modulator of inflammatory responses. Roles for fatty acids in inflammation are well described. Omega-6 fatty acids are precursors of enzymatically synthesised oxidised derivatives (e.g. prostaglandins and leukotrienes) that have well known roles in inflammation. Omega-3 fatty acids, especially EPA and DHA, act through several interconnected mechanisms to modulate inflammatory process and to actively initiate resolution of inflammation. Effects of saturated fatty acids (SFAs) in inflammation are less well explored. In vitro studies show that some SFAs including lauric, myristic and palmitic acids can directly activate inflammatory cells through toll-like receptor-4. Macrophages taken from mice fed high fat diets rich in saturated fat (lard or hydrogenated coconut oil) show exaggerated inflammatory cytokine responses (e.g. TNF-alpha gene expression and production) when stimulated with bacterial endotoxin, an effect that may relate to altered membrane composition. Epidemiological studies in humans show a positive association between circulating individual (myristic, palmitic) and total SFAs and some markers of low-grade inflammation like plasma IL-6. This lecture will review the literature on SFAs and inflammation and the mechanisms that seem to underlie the observed effects.

Considerations for Using Food Ingredients Containing Saturated Fats from a Food Ingredient Manufacturing Perspective. B. Flickinger, Archer Daniels Midland Company, USA.

Saturated fats have become of renewed importance for functional purposes in applications needing solid fat structure and stability given the move away from the use of *trans* fatty acids. This renewed importance has renewed the focus on individual saturated fatty acids from a functional and nutritional perspective. Manufacturers of food ingredients play a key role in delivering edible fats and oils ingredients which meet the above demands. Each edible fat or oil delivers each class of fatty acid -- saturated, monounsaturated and polyunsaturated -- in which the proportion each class plays a role in determining the functional and nutritional properties. As such, the emphasis of this presentation will focus on the balance of supply and application of technology with functional and nutritional considerations faced by manufacturers of edible fats and oils ingredients.

H&N 3.1/BIO 3: Biomodifications, Biomechanisms, and Biosafety

This session is sponsored in part by DuPont Nutrition & Health, Johnson & Johnson, and Oilseeds & Bioscience Consulting

Chairs: R.F. Wilson, Oilseeds & Bioscience Consulting, USA; and M. Picklo, USDA, ARS, USA

Dietary Seed Oil Effects on Kidney Oxylipins Reveal Surprising Effects of Fatty Acids. H. Aukema[1,2], [1]University of Manitoba, Canada, [2]Canadian Centre for Agri-Food Research in Health and Medicine, Canada.

Oxylipins are biologically active products of polyunsaturated fatty acids [e.g. eicosanoids derived from arachidonic acid (AA)]. Interestingly, the oxylipins formed from the two main polyunsaturated fatty acids in dietary seed oils, namely linoleic (LA) and a-linolenic acid (ALA), are almost completely unexplored. However, they are present in large amounts in animal tissues, they can have bioactivity similar to the other oxylipins, and diet significantly alters these oxylipins. In obesity-associated nephropathy, the oxylipins derived from ALA correlated with protection from the development of nephropathy. Furthermore, although the level of dietary LA did not change the levels of AA, the levels of AA (and LA) oxylipins were altered, demonstrating that fatty acid level does not necessarily predict potential biological effects. In another renal disease, the protective effect of dietary flax oil on disease progression was associated with amelioration of oxylipin abnormalities. Interestingly, despite the belief that ALA is poorly converted to docosahexaenoic acid (DHA), and the reduced DHA levels were not restored by flax feeding, dietary ALA was converted to DHA in sufficient amounts to restore the lowered DHA oxylipins in this disease. This suggests that the conversion of ALA to DHA is greater than is reflected by fatty acid levels.

Characterization of *Brassica napus* Type-1 Diacylglycerol Acyltransferase Variants Produced Through Directed Evolution. Y. Xu, G. Chen, and R.J. Weselake, University of Alberta, Canada.

Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of *sn*-1,2-diacylglycerol to produce triacylglycerol (TAG) and CoA. The level of DGAT activity during seed development appears to have a substantial effect on the flow of carbon into seed oil. Previously, directed evolution approach was used to generate an assortment of *Brassica napus* C.DGAT1.a (BnaC.DGAT1.a) variants which resulted in increased TAG content when recombinant forms of the enzymes were produced in *Saccharomyces cerevisiae* strain H1246. In the current study, microsomes were prepared from H1246 yeast lines, producing various recombinant Bna.C.DGAT1.a variants, at the mid-log phase of growth. The microsomal fractions were then analyzed for DGAT activity using radiolabeled substrate and BnaC.DGAT1.a polypeptide using Western blotting. The results of these assays indicated that the BnaC.DGAT1.a variants could be divided into three groups: 1) enzymes with increased activity; 2) enzymes which exhibited increased polypeptide accumulation; and 3) enzymes which were activated along with exhibiting increased polypeptide accumulation. The most promising variant, which resulted in a 2.3-fold increase in TAG content in yeast relative to the native form of the enzyme, belongs to category 3.

***In vivo* and *in vitro* Evidence for Biochemical Coupling of Reactions Catalyzed by Lysophosphatidylcholine Acyltransferase and Diacylglycerol Acyltransferase.** X. Pan[1], S. Stymne[2], J. Zou[3], X. Qiu[4], G. Chen[1], M. Kazachkov[3], I. Lager[2], M.S. Greer[1], and R.J. Weselake[1], [1]University of Alberta, Canada, [2]Swedish University of Agricultural Sciences, Sweden, [3]National Research Council Canada, Canada, [4]University of Saskatchewan, Canada.

Flax (*Linum usitatissimum* L.) seed oil is highly enriched in a-linolenic acid (ALA).

Phosphatidylcholine (PC) is the major site for ALA synthesis and thus efficient mechanisms are required to channel ALA from PC into triacylglycerol (TAG). The PC de-acylation reaction catalyzed by the reverse action of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) could potentially transfer ALA produced on PC directly into the acyl-CoA pool making this polyunsaturated fatty acid (PUFA) available for the diacylglycerol acyltransferase (DGAT)-catalyzed reaction for TAG production. Firstly, *in vivo* experiments showed that co-expressing flax *DGAT1* and *LPCAT* in a yeast quintuple mutant significantly increased 18-carbon PUFA in TAG with a concomitant decrease of 18-carbon PUFA in phospholipid. Secondly, *in vitro* experiments further showed that yeast microsomes

containing both DGAT1 and LPCAT were able to transfer [^{14}C]-labeled linoleoyl or linolenoyl moiety at a higher rate than oleoyl moiety from the *sn*-2 position of PC to TAG. Together, our data support the hypothesis of biochemical coupling of the LPCAT reverse reaction with the DGAT1 forward reaction for the incorporation of ALA into TAG. This process represents a possible mechanism for enriching TAG in PUFA during seed development in flax.

Preparation of High-purity DHA from Microalgae Oil in a Packed Bed Reactor via Two Step Lipase-catalyzed Esterification. E.J. Lee[1], D.S. No[1], M.W. Lee[1,2], and I. Kim[1], [1]Korea University, Republic of Korea, [2]Ilshinwells, Republic of Korea.

High-purity docosahexaenoic acid (DHA) was produced successfully in a packed bed reactor via two-step lipase-catalyzed esterification using the fatty acid from microalgae (from Crypthecodinium cohnii) and ethanol as substrates. Lipozyme RM IM from Rhizomucor miehei was employed as a biocatalyst. For the first reaction, several parameters including temperature, molar ratio of the substrates (fatty acid to ethanol), and water content in the substrate mixture (based on the total substrate weight) were investigated as a function of the residence time. A temperature of 40^{o}C, a molar ratio of 1:4, and a water content of 0.6% were selected as optimum conditions. Under these conditions, the maximum DHA concentration of over ca. 90% was achieved in the residual fatty acid fraction with an 89% yield. For the second reaction, the residual fatty acid separated from the first reaction product was used as a substrate. The optimum condition of the first reaction was used for the second reaction. After the second reaction, a DHA concentration of 100% was achieved in the residual fatty acid with a 94% yield.

Solvent-induced 7*R*-Dioxygenase Activity of Soybean 15-lipoxygenase-1 in the Formation of Omega-3 DPA-derived Resolvin Analogs. E.P. Dobson, C.J. Barrow, and J.L. Adcock, Deakin University, Australia.

The resolvin family contains important anti-inflammatory and pro-resolution compounds enzymatically derived *in vivo* from the omega-3 polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). More recently, docosapentaenoic acid (DPA) has emerged as another potentially important precursor in the biological production of resolvin compounds.

In this work we have used medium engineering to develop a simple method for the controlled synthesis of two di-hydroxylated diastereomers of DPAn-3 catalyzed by soybean 15-lipoxygenase-1 (15-sLOX-1) in the presence of short chain *n*-alcohols, including methanol, ethanol and propan-1-ol. The complete structures of the two major products – 7*S*,17*S*- and 7*R*,17*S*-dihydroxy-DPAn-3 have been elucidated using various chromatographic and spectroscopic analyses, including chiral HPLC, UV-visible, FTIR, and NMR spectroscopy. The alcohol-dependant *R*-dioxygenase activity of 15-sLOX-1 with mono-hydroperoxide intermediate substrates has also been demonstrated with other biologically relevant PUFAs, including DHA, EPA and arachidonic acid. The developed method has applications in the production of closely related isomers of naturally occurring resolvins and protectins, demonstrating the versatility of 15-sLOX-1 as a biocatalyst.

Effect of Dietary Lysophospholipids Containing n-3PUFAs on Serum and Liver Lipids Contents in Rats. R. Hosomi[1], K. Miyauchi[1], K. Fukunaga[1], Y. Inoue[2], T. Nagao[3], M. Yoshida[1], and K. Takahashi[4], [1]Kansai University, Japan, [2]Lipid Lab., Japan, [3]Osaka Municipal Technical Research Institute, Japan, [4]Hokkaido University, Japan.

The EPA and DHA have beneficial health properties, *i.e.,* decrease in serum triglyceride (TAG) and anti-platelet aggregation. Bioavailability of n-3PUFAs depends on their chemical form, such as TAG, ethyl-ester, fatty acid, and phospholipid. Superior bioavailability has been suggested for phospholipid containing n-3PUFAs. Currently, no information is available concerning the effect of lysophospholipids (Lyso-PLs) containing n-3PUFAs on lipid metabolism. In this study, we investigated the effects of Lyso-PLs containing n-3PUFAs on serum and liver lipids contents in rats. Lyso-PLs containing n-3PUFAs were prepared from squid meal, which is fisheries byproduct. Groups of male Wistar rats were fed AIN93G diet containing soybean oil (SO, 7%), TAG containing n-3PUFAs (2.0%) + SO (5.0%), and Lyso-PLs containing n-3PUFAs (2.0%) + SO (5.0%). The following indicators were assayed as indexes of lipid metabolism: TAG and cholesterol in serum and liver, fecal cholesterol, bile-acid excretion. Serum TAG and cholesterol contents decreased significantly in the group fed Lyso-PLs containing n-3PUFAs as compared with TAG containing n-3PUFAs, which had the same contents of n-3PUFAs as the Lyso-PLs diet. Therefore, Lyso-PLs containing n-3PUFAs has the

possible to be useful functional food materials.

Effect of Feeding DHA as Phospholipid, Triacylglycerol, or Both on DHA Concentration of Brain Regions, Liver, and Serum Lipids. A.P. Kitson[1], A. Berger[2], and R.P. Bazinet[1], [1]University of Toronto, Canada, [2]Arctic Nutrition, Norway.

DHA is important for neurological function, but the form of dietary DHA most bioavailable to the brain is not agreed upon. There is evidence that DHA in phospholipids (PL) is more bioavailable then triacylglycerol (TAG), however previous studies comparing PL- and TAG-DHA only examine acute brain uptake after one dose and have not examined differences in metabolism. This study compared DHA as TAG-rich fish oil (FO), PL-rich caviar PL concentrate (PLC), or a mixture of both (FO+PLC) on brain, liver, and serum DHA. After 11 weeks on a low omega-3 diet, male rats received 2% fatty acids as DHA as FO, PLC, or FO+PLC or an olive-oil (OO) supplemented diet for 4 weeks. DHA was higher in total lipids of cortex, cerebellum, hippocampus, brainstem, striatum, and rest of brain as well as liver in all DHA supplemented groups relative to OO controls with no significant differences between supplemented groups. Similarly, DHA-supplemented groups had higher DHA in serum PL, TAG, non-esterified fatty acids and cholesterylesters relative to OO with some evidence of higher serum DHA in FO+PLC-supplemented rats relative to other DHA groups. All forms of DHA appear equally effective at increasing tissue DHA, while combined PL+TAG-DHA may target serum DHA.

An Efficient Gene Targeting and Molecular Breeding in Oil-producing Fungus *Mortierella alpina* with Deletion of *lig4* Gene for Non-homologous End Joining. H. Kikukawa[1], E. Sakuradani[1,2], A. Ando[1], S. Shimizu[1], and J. Ogawa[1], [1]Kyoto University, Japan, [2]University of Tokushima, Japan.

The oil-producing zygomycete *Mortierella alpina* 1S-4 is known to accumulate beneficial polyunsaturated fatty acids (PUFAs), such as arachidonic acid (ARA) and eicosapentaenoic acid (EPA). To achieve high production of valuable PUFAs by metabolic engineering, an efficient gene deletion system, such as gene targeting, is necessary for PUFAs production by this fungus. Here, to develop an efficient gene targeting in *M. alpina* 1S-4, we identified the *lig4* gene encoding DNA ligase 4 involved in non-homologous end joining (NHEJ) on genomic double strand breaks repair, and then constructed *?lig4* strain from *M. alpina* 1S-4. The replacement in the *?lig4* strain was improved from 3% to 67% of gene targeting efficiency. In addition, the *?lig4* strain showed no defect in vegetative growth and formation of spores. Furthermore, dihomo-?-linolenic acid (DGLA)-producing strains were constructed with deletion of *?5-desaturase* (*?5ds*) gene encoding a key enzyme of bioconversion of DGLA to ARA using the *?lig4* strain as a host strain. DGLA composition of the strains reached about 40%, and in contrast, ARA composition of the strains decreased. From these results, the *?lig4* strain from *M. alpina* 1S-4 is available for general strain of metabolic engineering.

H&N 4: Vitamin D: Basic, Clinical, and Translational Research

Chairs: H.A. Durham, University of California, Los Angeles, and Pennington Biomedical Research Center, USA; and M.L. Drewery, Louisiana State University, USA

This session has been cancelled.

H&N 5: General Health and Nutrition

Chairs: M. Torres-Gonzalez, Dairy Research Institute, USA.

Cosolubilization of DHA and Curcumin as Synergistic Nutraceuticals as Anti-inflammation Nutraceuticals. N. Garti, K. Ozelevski, and A. Aserin, Hebrew University of Jerusalem, Israel.

Many nutraceuticals that are water insoluble are very difficult to transport across the guts membranes to the blood stream. For such nutraceuticals we need vehicles or carriers to solubilize them and to guide them to the jejunum and facilitate their transport across the membrane. Micremulsions and especially dilutable microemulsions are capable of solubilizing various water insoluble and lipophilic nutraceuticals min very high solubilization capacity.

In this study we attempted to solubilize two in one nanodroplet and test their ability to stay at the interface of the nanodroplets as stable formulation and to release the drugs across the membrane in a consecutive manner.

The two nutraceuticals that were selected were curcumin along with either ethyl ester of DHS (EE-DHA) or triglyceride DHA (TAG-DHA).
We studied the solubilization capacity and its effect on the structure variations of the vehicles when each was solubilized alone and in all possible combinations.

Radical-induced Lipid Transformations Under Hypoxic Condition. A.G. Lisovskaya, A. Kulinkina, and O. Shadyro, Belarusian State University, Belarus.

The importance of free radical fragmentation reactions was pointed out for the first time in our studies on radiation-induced transformations of lipids under hypoxic conditions. We have shown that under such conditions the fragmentation of carbon- or nitrogen-centered radicals formed from the starting lipids takes place. In the case of hydroxyl-containing glycerophospholipids, such as cardiolipin, the fragmentation resulted in the formation of phosphatidic acid, which is widely implicated in regulating the mTOR-mediated signals that promote cancer cell survival. Free radical fragmentation of galactocerebrosides led to formation of ceramides, which are known to be signaling molecules that play an important role in cell apoptosis processes. Furthermore, our studies were first to identify bioactive 2-hexadecenal among the major products of free radical fragmentation of sphingolipids. Thus, our studies have revealed important free radical reactions taking place in lipids, which give bioactive molecular products capable of interfering with vital biochemical processes. The facts established in these experiments should be taken into account while performing research work aimed at the development of novel therapeutics intended to be used for treatment of diseases associated with the activation of free radical processes in living organisms.

Physical and Oxidative Stability of Fish Oil-in-Water Emulsions Stabilized with Fish Protein Hydrolysates. P.J. García Moreno[1], A. Guadix[1], E.M. Guadix[1], and C. Jacobsen[2], [1]University of Granada, Spain, [2]Technical University of Denmark, Denmark.

To successfully develop food enriched with omega-3 PUFA, lipid oxidation of these highly unsaturated fatty acids must be prevented in order to avoid both the loss of nutritional value and the formation of unpleasant off-flavors. In this sense, one of the strategies developed to protect these oxidative unstable lipids when incorporating them into food is the employment of delivery systems such as fish oil-in-water emulsions. In these systems, the emulsifier determines the structure and thickness of the interfacial layer, which is the place of contact between lipids and prooxidative components.[1] Thus, emulsifiers exhibiting also antioxidant activity are preferred in order to reduce lipid oxidation. In this context, fish protein hydrolysates could be good candidates to be employed as emulsifier due to their recognized emulsifying and antioxidant properties.[2]

In the light of the above, the objective of this work was to investigate the influence of sardine fish protein hydrolysates on the physical and oxidative stability of fish oil-in-water emulsions. For that purpose, four hydrolysates with different degree of hydrolysis (3, 4, 5 and 6%) and obtained by using subtilisin were evaluated. Firstly, the antioxidant capacity of the hydrolysates was tested by in vitro assays (DPPH, chelating activity and reducing power). Secondly, the physical stability of the emulsions was determined by measuring their viscosity, zeta potential and droplet size. Finally, in order to determine the oxidative stability of the emulsions, analysis of peroxide value, tocopherols content and volatile oxidation products were carried out. The stable emulsions produced are susceptible

to be used directly as omega-3 delivery systems in order to produce fortified food.

[1]Hunt, J.A., & Dalgleish, D.G. (1994). Adsorption behaviour of whey protein isolate and caseinate in soya oil-in-water emulsions. Food Hydrocolloids 8: 175-187.

[2]Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (Selaroides leptolepis) as influenced by the degree of hydrolysis and enzyme type. Food Chemistry 102: 1317–1327.

Enhance Intestinal Lymphatic Transport of Lipophilic Bioactive Food Components by Nanoemulsion Delivery System. M. Yao[1], D.J. McClements[1,2], and H. Xiao[1], [1]University of Massachusetts Amherst, USA, [2]King Abdulaziz University, Saudi Arabia.

Lipid-based formulations can effectively enhance the bioavailability of highly lipophilic components by improving their absorption in the gastrointestinal tract while facilitating intestinal lymphatic transport. Herein, we studied the effects of triglycerides-based nanoemulsion delivery systems with different fatty acid chain lengths on the bioavailability of a highly lipophilic flavonoid, 5-demethylnobiletin (5-DN). 5-DN was encapsulated in medium chain triglycerides (MCT) or canola oil based nanoemulsion. They were subject to a simulated gastrointestinal digestion model. Finally, the mixed micelle phase was applied to Caco-2 monolayer cell model that mimics intestinal absorption. Higher bioaccessibility of 5-DN was found in MCT nanoemulsion than canola nanoemulsion, 13% vs.7% respectively. However, only 30% 5-DN crossed Caco-2 monolayer while half of them were metabolized for MCT nanoemulsion, up to 60% 5-DN and only 10% were metabolized in canola nanoemulsion. Results also demonstrated more lipid droplets and chylomicrons were formed by canola nanoemulsion, which were responsible for transportation of 5-DN to the lymph. In conclusion, LCT-based emulsion was more potent in enhancing the bioavailability through increased lymphatic transport.

Stabilization Activity and Rancidity Inhibition of Phenolic Compounds from Oregano *(O. Vulgarea)*, Rosemary *(R. Oficianalis)*, and Lemon Grass *(C. Citratus)*. N.R. Navarro, Nueva Ecija University of Science and Technology, Philippines.

Consumer's preferences on organically derived products continue to grow so as the efforts in combating diseases caused by free radicals from food additives. These organically derived products possess a great possibility of replacing synthetic food additives.

The present study aimed to evaluate the stabilization and rancidity inhibition property of locally available herbs and spices. Three common herbs and spices in the Philippine were tested, Oregano, Rosemary, and lemon grass.

Their stabilization and preservation capacity were tested using GC-MS analysis and determination of Oxidative Stability Index utilizing Rancimat instrument. Results have shown that after the characterization of fatty acids present both on experimental and control group, the fatty acid content were at the same level. Strengthening the results of GC-MS is the Oxidative Stability Index that showed that the OSI of oil products containing the plant extracts have longer stability than that of control group. The OSI of oil containing plant extracts were at 25 hours and for control groups were at 8 hours respectively, proving the strong antioxidant capacity of the plant extracts.

H&N 5.1/BIO 5.1/SCC: Lipid Oils and Skin Health

This session is sponsored in part by Johnson & Johnson

Chairs: K. Mahmood, Johnson & Johnson Consumer, USA; T.A. McKeon, USDA, ARS, WRRC, USA; and K. Dobos, Society of Cosmetic Chemists/Sun Chemical Corp., USA

The Role of Lipids in Skin Physiology. A. Pappas, Johnson & Johnson, USA.

The skin is the largest organ of the human body, which serves functions in thermoregulation, protection, metabolism and in sensory. Various lipids are fundamental for normal skin functions and are unusual as they are not found anywhere else in the human body. Sapienic acid, triglycerides, waxes and squalene are secreted by glands imbedded in the human skin and are deposited on the surface of the skin. These lipids contains an unusual mixture of fatty acids. Recently, the importance of all these lipids was further validated in animal models where the biosynthesis of fatty acid esters, triglycerides, long chain fatty acids and oleic acid has been impaired. All these animal studies together with recent reports on the effect of the dietary fat on skin, further demonstrate the importance and essential role of fatty acid metabolism in normal skin physiology. Vegetable oils, fruit extracts and their components: fatty acids, tocopherols, polyphenols are constantly used in a wide variety of topical consumer products and their effects on skin care and on dermal health had been seriously underestimated.

New Insights into the Role of Polyunsaturated Fatty Acids in Skin Physiology and Pathology. H. Gallagher, I.A. Guschina, D. Ramji, and J.L. Harwood*, Cardiff University, UK.

Ever since skin defects revealed the need for essential fatty acids in the diet, the important role of PUFA in the biochemistry of epidermal layers has been acknowledged. Interest has also included disease such as atopic eczema and psoriasis where certain dietary PUFA can alleviate symptoms in both animal models and human subjects.

One of the PUFAs tested is the n-6 acid, dihomo-gamma-linolenic acid (DGLA). Like n-3 PUFAs such as EPA or DHA, DGLA can be metabolised to anti-inflammatory compounds – in this case, prostaglandin E_1 or 15-hydroxyeicosatrienoic acid. Moreover, DGLA has been suggested as a compound of key importance in diseases such as atopic dermatitis. We have studied the metabolism and effects of DGLA using a number of tissue culture systems. DGLA is taken up rapidly, esterified into a variety of membrane lipids and has rapid effects on a number of inflammatory mediators. It is oxidised by both cyclooxygenase and lipoxygenase enzymes whose products are readily detected by MS. At the same time, its provision reduces the relative amount of its major metabolic product, arachidonate. This may be important since arachidonate is an n-6 PUFA which produces mainly inflammatory eicosanoids.

Biosynthesis and Skin Health Applications of Antimicrobial Glycolipids. D.K.Y. Solaiman and R.D. Ashby, USDA, ARS, ERRC, USA.

Microbial-produced glycolipids (MGLs) such as sophorolipids (SLs), rhamnolipids (RLs), and mannosylerythritol lipids (MELs) are amphiphilic molecules, and thus have been widely explored for use as surfactants/detergents, emulsifiers, and lubricants. A major hindrance to their widespread commercial adoption is the higher prices in comparison to their non-renewable petroleum-based counterparts. To overcome this, research abounds in developing production systems that will lead to lower production costs. On top of that, many studies have been and continue to be conducted to find and explore any value-added properties possessed by these molecules which could help justify the higher costs of MGLs. This paper will first survey the current advances in production trends and skin health applications (e.g., antimicrobial, woundhealing promotion, fibroblast rejuvenation, emollient, etc.) of MGLs. We will then present our lab's research on using surplus agricultural byproducts as inexpensive feedstocks in the fermentative production of MGLs, and provide evidence for SLs as antibacterial agents particularly by demonstrating anti-acne activity against the causative bacterium (*Propionibacterium acnes*) when immobilized on biopolymer films.

Meadowfoam (*Limnanthes alba*) Natural Products Inhibit Matrix Metalloproteinases in Human Keratinocytes: Relevance to Skin Health. C.L. Miranda, R.L. Reed, A.K. Indra, and J.F. Stevens*, Oregon State University, USA.

Meadowfoam (*Limnanthes alba*) is an oilseed crop which is grown in the Willamette Valley of Oregon. Meadowfoam seed oil has commercial value

as an ingredient of cosmetic products. A waste product of the oilseed extraction, the seed meal is a rich source of the glucosinolate, glucolimnanthin. We developed a patented procedure for enzymatic conversion of glucolimnanthin into the bioactive products, 3-methoxybenzyl isothiocyanate (MBITC) and 3-methoxyphenyl acetonitrile (MPACN), by fermentation of meadowfoam seed meal. We investigated the anti-inflammatory potential of these products in human skin cells with the aim to explore their use as meadowfoam oil additives to improve skin health. Skin keratinocytes overexpress pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs) in response to stimulation by UV light, chemical irritants and infection. We found that an extract of fermented meadowfoam seed meal and especially MBITC (at 1 µM), but not MPACN, inhibit MMP-9 protein expression and activity in stimulated HACAT keratinocytes without signs of cytotoxicity. These findings demonstrate the beneficial effects of a fermented meadowfoam seed meal extract and MBITC as additives to cosmetic oils for the prevention of skin inflammation through inhibition of MMPs.

Cosmetic Applications of Castor Oil and Its Derivatives. T.A. McKeon and X. He, USDA, ARS, WRRC, USA.

Castor oil is unique - it contains 90% of the hydroxy fatty acid ricinoleic acid. The hydroxy group imparts unique physical, chemical and physiological properties on castor oil. While best known for its laxative effect, castor oil derived products include lithium grease, engineering nylons and cosmetics. Castor oil is a common constituent of lipsticks and cosmetic creams. Derivatives of castor oil also are effective as anti-microbials, and may be used to preserve cosmetics from microbial deterioration. The castor oil seed contains potent allergens and the toxin ricin in the protein fraction. We evaluated the possibility that ricin might be present in castor oil. We obtained samples of castor oil subjected to different levels of processing and from commercial sources. After extraction of the oil with phosphate-buffered saline, we tested the aqueous extracts for ricin content. We detected 35ng/ml of ricin (lethal dose ˜2-5µg/kg) in samples derived from the cold-pressed castor oil supplied and from a commercially purchased sample. However, no ricin was detected in USP grade castor oil, neutralized or hydrolyzed castor oil. The presence of ricin in the cold-pressed oil could indicate that allergen is also present, indicating that use of cold-pressed castor oil for ingestion or topical application be avoided.

Production of Structured Phospholipids using Phospholipase and Lipase. S.H. Yoon, Woosuk University, Republic of Korea.

The transesterification abilities of microbial phospholipase A2 and lipase in organic solvents were studied. Phosphatidylcholine and caprylic acid were transesterified by incubation in organic solvents using phospholipase A2 and lipase. Caprylic acid was incorporated into the *sn*-1 and -2 positions at a rate of 87.7% using phospholipase A2 in hexane, and 36.7% using lipase in diethyl ether. Higher acyl migration into *sn*-2 was observed in diethyl ether than in hexane during transesterification using lipase, however, there was no substantial difference in the caprylic acid content at the *sn*-2 position. Acyl migration during transesterification in methanol was lower than in other organic solvents.

Preparation of Highly Purified Pinolenic Acid from Pine Nut Oil via Three-step Lipase-catalyzed Esterification. H.J. Kim[1], T.T. Zhao[1], D.S. No[1], C.T. Kim[2], and I.H. Kim[1], [1]Korea University, Republic of Korea, [2]Korea Food Research Institute, Republic of Korea.

Pinolenic acid (PLA) from pine nut oil was successfully enriched by three-step lipase-catalyzed esterification. The fatty acids present in pine nut oil were selectively esterified with ethanol using Lipozyme RM IM from *Rhizomucor meihei* as a biocatalyst and PLA was enriched in the fatty acid fraction. The optimum conditions of molar ratio of the substrate (fatty acid to ethanol) and temperature were 1:7 and 25°C, respectively. There was no significant effect in the enrichment of PLA when water was added in reaction mixture. The same protocol and optimum conditions were employed for second and third step lipase-catalyzed esterifications. For first step lipase-catalyzed esterification, PLA was enriched up to 41.6mol% from an initial value of 13.5mol% in the pine nut oil. Using PLA enriched fatty acid obtained from first step as a substrate, PLA was enriched up to 68mol% via second step lipase-catalyzed esterification. Consequently, a maximum PLA content of ca. 78mol% was obtained via third step lipase-catalyzed esterification.

H&N-P: Health and Nutrition Poster Session

Chairs: H.A. Durham, University of California, Los Angeles, and Pennington Biomedical Research Center, USA; and M.L. Drewery, Louisiana State University, USA

1. **Inhibition of *in vitro* Acetylcholinesterase Activity by Hemp Seed Protein Hydrolysates.** S.A. Malomo ***(Honored Student Award Winner and Health and Nutrition Division Student Excellence Award Winner)*** and R.E. Aluko, University of Manitoba, Canada.

Hemp seed protein (HSP) and its hydrolysates (HPHs) are increasingly attracting global attention as health promoting agents due in part to their non-existence as priority-labelled allergenic proteins. Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh); therefore, excessive activity can reduce or terminate signal transmission to the brain and result in Alzheimer's disease (AD) development. AChE-inhibitors are therefore, needed to increase ACh concentration in the brain. This study aimed to examine the *in vitro* AChE-inhibitory properties of HPHs from HSP. HSP isolate (HPI) was hydrolyzed using three different proteases (*pepsin, papain* and *alcalase*) at different enzyme concentrations (1-4%). Results of amino acid composition showed that HPHs had higher negatively charged amino acid (39.62-40.18%) and hydrophobic amino acid (26.08-26.99%) than HPI (39.41% and 26.03% respectively). *In vitro* AChE-inhibition results showed that 1% *pepsin* HPH had the strongest ($p<0.05$) activity (IC50; 0.06mg/ml) while the least was 4% *alcalase* HPH (IC50; 0.12mg/ml). Gel permeation chromatography showed that most of the peptides were of low molecular weight and within the 300-9500Da size range. We conclude that HPHs may be used as natural AChE-inhibitory agents with potential health benefits in the treatment of AD.

2. **Effects of Life-long Diets of Common Unsaturated Fatty Acids from Vegetable Oils on Lifespan and Oxidation in a Caenorhabditis Elegans Model.** B. Fang[1], F. Ren[2], Y. Wang[1], and X. Zhou[2], [1]Academy of State Administration of Grain, China, [2]China Agriculture University, China.

It is now generally accepted that the type of dietary fat that is consumed plays a significant role in health and disease. As an important dietary component, vegetable oils contain many UFAs, the major of which contain all or some of the 18-carbon fatty acid series of oleic acid (OA, C18:1, n-9 MUFA), linoleic acid (LnA, C18:2, n-6 MUFA) and a-linolenic acid (ALA, C18:3, n-3 PUFA). Here in this study, we evaluate the effects of dietary oleic acid (OA), linoleic acid (LnA) and linolenic acid (ALA) on lifespan and physical activities of *Caenorhabditis elegans*. At doses of 0.5mg OA/plate, 0.1mg LnA/plate and 0.5mg ALA/plate, the lifespan of *Caenorhabditis elegans* was extended by 10.49%, 14.17% and 8.47%, respectively ($p<0.05$). At these doses, LnA and ALA significantly inhibited growth, pharyngeal pumping, reproduction and respiration ($p<0.05$), while OA did not influence these physiological activities ($p>0.05$). Furthermore, OA increased the activity of three antioxidases. In conjunction with the results for *eat-2* and *sod-2* mutated *C. elegans*, it was found that LnA and ALA extended the lifespan of *C. elegans* by activating the caloric restriction mechanism whereas OA worked via a hormesis mechanism by activating the antioxidation defense system.

3. **Comprehensive Lipidomics of Plasma, Erythrocyte, and Whole Blood of Humans with Low, Intermediate, and High n-3 PUFA Status.** J.A. Henao, R. Smith, and K. Stark, University of Waterloo, Canada.

Lipidomic analyses of blood lipids and the response to n-3 PUFA intervention have been examined, but an assessment of plasma, erythrocyte (RBC) and whole blood (WB) across individuals with different n-3 PUFA status is needed. The lipidomic profiles of venous WB, plasma, and RBC were determined using ultra-high performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS). Samples from individuals with low, medium, and high n-3 PUFA status (n=3/group) as determined by gas chromatography were used. WB provided the most comprehensive lipidomic profile. Phosphatidylethanolamine and phosphatidylserine species were abundant in RBC but not plasma, while the opposite was true for triacylglycerols and cholesteryl esters. In general, an increase in n-3 PUFA concentrations was reflected by increases in 16:0/22:6, 18:0/22:6, 16:0/20:5, and 18:0/20:5 in phosphatidylcholines and phosphatidylethanolamines but not phosphatidylserines. In conclusion, WB lipidomics was the most informative approach due to the comprehensive nature of the sample, combined with

the discrimination ability of UHPLC-MS/MS. The increases in n-3 acyl species by lipidomics were consistent with increases in n-3 PUFA concentrations by gas chromatography. This work provides the foundation for the use of complex lipid acyl species as biomarkers for n-3 PUFA status.

4. **Risk Factors for Age-related Macular Degeneration Appear Early in Life Among Female College-aged Students.** A.V. Gaitán[1], A. Ocampo[1], C. Childress[1], M.L. Drewery[1], R. Pinkston[1], C.J. Lammi-Keefe[1,3], and H.A. Durham[2,4], [1]Louisiana State University, USA, [2]Pennington Biomedical Research Center, USA, [3]Louisiana State University AgCenter, USA, [4]California State University, Los Angeles, USA.

AMD is the leading cause of vision loss, with females at highest risk. While the reasons for this are not fully understood, dietary docosahexaenoic acid (DHA) and lutein and zeaxanthin (L&Z) are documented to reduce AMD risk. We collected health histories and documented food intake with 24-hour dietary recalls from 247 college-aged participants. Macular pigment optical density (MPOD), a measure of thickness of the macula and possible predictor for risk of AMD, was measured with a macular metrics densitometer. Gender, age, body mass index, ethnicity, and eye color were recorded. Using analysis of variance, females (n=150) consumed less DHA compared to males (n=97) [Mean (±SE): females=69±21mg, males=158±26 mg (P<0.01)] and less L&Z [Mean (±SE): females=1,343±295µg, males=2,862±368µg (P<0.01)]. Females had lower MPOD than males [Mean (±SE): females=0.31±0.01, males=0.36±0.02 (P=0.03)]. MPOD was positively correlated with amount of seafood high in DHA consumed weekly (r=0.14, P=0.02). In summary, college-aged females had lower MPOD than males and consumed significantly less DHA and L&Z from diet and supplements than males. We conclude that eating seafood high in DHA and foods with L&Z early in life may protect against AMD for females later in life.

5. **Conjugated Linoleic Acid Increases Voluntary Activity and Muscle Mass via Mitochondrial Biogenesis in Adult Onset Inactivity-induced Obese Mice.** Y. Kim[1], D.J. Good[2], and Y. Park[1], [1]University of Massachusetts Amherst, USA, [2]Virginia Polytechnic Institute and State University, USA.

Our study demonstrates how conjugated linoleic acid (CLA) improves physical activity levels and increases muscle mass by determining the molecular targets on mitochondrial biogenesis in nescient basic helix-loop-helix 2 knock-out (N2KO) mice, a unique adult onset inactivity-induced obesity model. Control or 0.5% CLA containing diet was fed to 4-week-old female N2KO and wild-type mice for 10 weeks. CLA-fed mice in both genotypes showed significant increased voluntary movement and gastrocnemius muscle mass. CLA treatment up-regulated peroxisome proliferator-activated receptor gamma co-activator 1a (PGC-1a), a master regulator of mitochondrial biogenesis, via AMP-activated protein kinase (AMPK) activation in N2KO animals. Mitochondria biogenic molecular markers, peroxisome proliferator-activated receptor-d (PPARd), mitochondrial transcription factor A (Tfam) and cytochrome c, in CLA-fed mice were significantly overexpressed compared to control animals. These observations were supported by in vitro model wherein 50 µM CLA isomers (cis-9, *trans*-11 and *trans*-10,cis-12) selectively activated PGC-1a-PPARd and PGC-1a-Tfam signaling cascades in C2C12 myotubes. These results suggest that CLA acts as a potential exercise-mimetic, resulting in increased voluntary activity and muscle mass.

6. **The Evaluation of Soybean ß-conglycinin on Glucose Metabolism in Wistar Rats by Oral ^{13}C-glucose Administration.** N. Inoue, A. Funayama, and I. Ikeda, Tohoku University, Japan.

Previously, we suggested that ß-conglycinin (ß-CG) prevents the development of type 2 diabetes through the improvement of carbohydrate metabolism and insulin sensitivity in GK rats. However, though serum glucose and insulin levels were significantly lower in the ß-CG group as compared with the casein group, there was no significant difference in OGTT. By these results, we hypothesized that OGTT gave stress to the experimental animals by continuous tail blood sampling. Therefore, we evaluated the effect of ß-CG on glucose metabolism in Wistar rats by oral 13C-glucose administration. Male Wistar rats were fed an AIN-93G diet containing casein or ß-CG for 1wk. As the result of respiratory gas analysis after oral 13C-glucose administration, the peak of 13C/12C rate appeared faster in the ß-CG group than in the casein group. Serum adiponectin level was significantly higher in the ß- CG-fed rats. Serum and hepatic TAG levels in the ß-CG-fed rats were significantly lower. In this study, we showed that the measurement of energy metabolism by oral 13C-glucose administration was an effective alternative evaluation to OGTT. Furthermore, we clarified that soybean ß-CG improved carbohydrate metabolism

through the preferential glucose consumption and the enhancement of adiponectin secretion.

7. **Effect of Black Raspberry Seed Oil on Inflammatory Status in Obese Mice Induced by High Fat Diet.** H.J. Lee, H. Jung, H. Cho, and K.T. Hwang*, Seoul National University, Republic of Korea.

Black raspberry seed (BRS) oil contains about 30% a-linolenic acid, an n-3 fatty acid. The objective of this study was to evaluate effect of BRS oil on inflammatory status in obese mice induced by high fat diet (HFD). Five-week old C57BL/6 mice were fed HFD consisting of 60% calories from fat. Control diet was made of 5% soybean oil and 5% corn oil of the total calories, and BRS oil diet was of 10% BRS oil. Proteins and mRNA involving inflammation were determined by western blotting and real-time PCR, respectively. NF-κB, phospho-NF-κB, phospho-I-κBα, COX-2, and TLR4 proteins were lower and I-κBκ protein was higher in the liver of the BRS oil diet fed mice than those in the control group. mRNA levels of pro-inflammatory markers (NF-κB, COX-2, TNFa, IL-1ß, IL-6, MCP-1, iNOS, and leptin) in the liver and adipose tissue of the BRS oil group were lower than in those of the control. Correspondingly, anti-inflammatory markers (IL-10, arginase 1, Mgl-1, Chi3l3, and adiponectin) were higher in the BRS oil group than in the control. Results of this study suggest that BRS oil, which is a good source of a-linolenic acid, may have anti-inflammatory effects on HFD-induced obese mice.

8. **Effects of Consuming Thermally Oxidized Soybean Oil on Tryptophan-kynurenine Metabolism Pathway.** L. Wang, D. Yao, G. Shurson, and C. Chen*, University of Minnesota, USA.

Consumption of thermally-stressed vegetable oils has been suggested as a contributing factor in various adverse metabolic effects including loss of appetite, diarrhea, growth retardation, elevated organ weights. However, the underlying mechanism of this association is not well understood. In this study, the influences of consuming heated soybean oil on the metabolic system were examined through the metabolomics-guided biochemical analysis. Fresh soybean oil (FSO) diet (contain 7% fresh oil), or heated soybean oil (HSO) diet (contain 7% heated oil) were given to C57/B6 mice for 4 weeks, respectively. Result showed that feeding HSO reduced growth performance and decreased serum level of triglycerides compared to FSO. Metabolomic analysis indicated that HSO treatment altered the homeostasis of free amino acids in serum and tissues. Among observed changes induced by HSO, the decrease of tryptophan level in serum was correlated to the increases of tryptophan metabolites in urine such as kynurenic acid and nicotinamide *N*-oxide, suggesting HSO might induce catabolic metabolism of tryptophan. Accordingly, some of key enzymes in the tryptophan-kynurenine pathway were induced by HSO treatment. All these observations warrant further investigation on the effects of thermally-stressed oils on tryptophan metabolism.

9. **Anti-inflammatory Effect of Black Raspberry Seed Oil in db/db Mice.** H.J. Lee, H. Jung, H. Cho, and K.T. Hwang*, Seoul National University, Republic of Korea.

Black raspberry seed (BRS) oil contains about 30% α-linolenic acid. The objective of this study was to evaluate anti-inflammatory effect of α-linolenic acid-rich BRS oil in db/db mice. Six-week old C57BL/KsJ-db/db mice were fed diets consisting of 16% calories from soybean oil (control), 8% from soybean and 8% from BRS oil (BRS 50%), and 16% from BRS oil (BRS 100%) for 10 weeks. Fatty acid composition of the livers of the mice was analyzed by GC. Proteins involved in inflammation were determined by western blotting. Arachidonic acid, known to cause the inflammatory responses, and α-linolenic acid, known to inhibit n-6 fatty acid-induced inflammation, accounted for 2.62% and 8.45% of the total fatty acids, respectively, in the liver of the BRS 100% group, which were significantly ($p<0.05$) different from those of the control (5.77% and 2.24%, respectively). I-κBα protein was significantly ($p<0.05$) higher in the liver of the BRS 100% group than that of the control. Correspondingly, TLR4 and COX2 proteins were lower in the adipose tissues of the two BRS groups than in the control, although not significant ($p>0.05$). Results of this study suggest that BRS oil may have anti-inflammatory effect in db/db mice.

10. **Characteristic Distribution of Metabolites in *Oryza sativa* Rice.** N. Zaima[1], Y. Yoshimura[2], Y. Kawamura[3], and T. Moriyama[1], [1]Kinki University, Japan, [2]Okayama Prefectural University, Japan, [3]Kyoto Women's University, Japan.

Rice contains various functional food factors that have an impact on our daily lives. Although it is important to analyze the distribution, the analysis of the spatial distribution of these compounds in foods using conventional technology was difficult. Previously, we established the method to clarify the

distribution of metabolites in rice by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). In this study, we investigated the distribution of metabolites in Rice. Rice seeds were freeze-embedded with 2% CMC, the rice sections were attached to adhesive film and sliced to 20-µm thickness using a cryostat. MALDI-IMS analysis was performed using a LTQ-XL linear ion trap mass spectrometer (Thermo Fisher Scientific). DHB was used as a matrix. The distribution of lysophosphatidylcholine (LPC), a-tocopherol, and a-tocotrienol can be successfully visualize in rice sections. Interestingly, the distribution of LPC was different among LPC molecular species. LPC (16:0) is ubiquitously present in the endosperm of rice. LPC (18:0) is localized in the core of the endosperm. In contrast, LPC (18:2) and LPC (18:1) are present in the outer region of the endosperm. Our data suggest that there are unknown domains defined by metabolite distribution in rice.

11. **Formation of Filled Hydrogel Beads: Impact of Emulsion Structure on Retention Behaviour.** B. Zeeb[1], A.H. Sabberi*[2], J. Weiss[1], and D.J. McClements[2], [1]University of Hohenheim, Germany, [2]University of Massachusetts Amherst, USA.

Filled hydrogel particles can be utilized as tailor-made encapsulation- and delivery systems. In the present study, the impact of the emulsification technique on the retention behaviour of filled alginate beads was investigated. A simple extrusion method was used to generate alginate beads filled with nanoemulsions, whereas curcumin was selected as a marker molecule to monitor the diffusion process. First, nanoemulsions composed of the same constitutes (medium chain triacylglycerides, Tween 60, curcumin, phosphate buffer) were prepared using either low or high energy homogenization, namely spontaneous emulsification and microfluidization. Second, nanoemulsions were mixed with alginate solutions and then subjected to hydrogelation by dripping the mixture into calcium solutions. Turbidity and spectrophotometrical measurements showed that the release rate of nanoemulsions prepared by spontaneous emulsification is higher, whereas diffusion was reduced with increasing alginate concentration. Higher release rates can be attributed to the presence of Tween 60 micelles acting as carriers for curcumin, thus, promoting the diffusion from the alginate beads into the surrounding phase. These results have important implications for the design of delivery systems to control the release of lipophilic bioactive components within filled hydrogel particles.

12. **Dietary Supplements For Brain Function Improvement: Are Labels Really Informative?** F.B. Pimentel, R.C. Alves, A.S.G. Costa, M.A. Nunes*, and M.B.P.P. Oliveira, University of Porto, Portugal.

Some neurodegenerative diseases severely affect cognitive functions. Affected patients undergoing medical treatment may benefit from dietary supplementation to improve their nutrient intake. Several amino acids are particularly important to prevent or delay the progress of such diseases. Some are precursors of important brain neurotransmitters like serotonin, dopamine, norepinephrine, crucial for mood, and behavior. The aim of this study was to screen the amino acids composition of commercial dietary supplements (*n*=8) for cognitive function improvement. The study focused the following compounds: arginine, 5-hydroxitryptophan, glutamine, aspartic acid, glutamic acid, phenylalanine, tyrosine, lysine, and methionine. Each sample was evaluated in triplicate, derivatized with dansyl chloride and analyzed by HPLC with fluorescence detection, according to Pimentel *et al.*

Most results were in accordance with the labeled information. Some samples presented superior amounts than the reported, possibly due to the presence of plant extracts and/or peptides, additional sources of amino acids in the formulations. One sample contained significantly lower amounts of aspartic acid and arginine than those described and other did not mentioned at all the amino acid composition in the label. This study warns to the need to reinforce quality control measures regarding these products in a consumer´s protection perspective. R. C. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

13. **Ready-to-use Carrots Stored Under Modified Atmosphere Packaging Rich in CO_2.** C. Barbosa[1,2], M.R. Alves[1,2], and M.B.P.P. Oliveira*[2], [1]Instituto Politécnico de Viana do Castelo (IPVC-ESTG), Portugal, [2]Universidade do Porto, Portugal.

The food industry aims to be competitive, productive and sustainable. Therefore the objective of the present work was to extend shelf life of ready-to-use carrots, minimally processed (MP) or

precooked (PC) in modified atmosphere packaging (MAP). PC and MP carrots were stored over 28 and 17 days, respectively. Gas composition inside the package was 0%O_2+40%CO_2+60%N_2 for PC and 10%O_2+45%CO_2+45%N_2 for MP carrots. Sensory characterization was used to obtain a qualitative and quantitative description of the organoleptic properties, a Quantitative Descriptive Analysis was performed and correlated to physicochemical quality parameters, such as antioxidant activity and bioactive compounds (phenols, flavonoids, carotenoids and anthocyanins), colour, and firmness monitored over storage. Principal component analysis (Autobiplots) and canonical correlations analysis were used to investigate how parameters correlate over the storage. Results showed only negligible losses in antioxidant capacity correlated to bioactive compounds content which variations did not present a clear tendency also confirmed by the sensory panel that did not found significant differences between samples over storage time. It was possible to conclude that the studied MAP conditions are adequate to preserve samples and advisable to implement on an industrial scale to improve logistic operations.

14. **Effects of Green Algae Feeding on Mouse Lipidome.** Y. Ma, W. Zhou, R. Ruan, G. Shurson, and C. Chen*, University of Minnesota, USA.

As a rich source of lipids, fatty acid content of algae has been extensively studied, but the impact of algae on the lipidomes of humans and animals is not well defined. In this study, a fresh water green algae (*Scenedemus spp.*) high in polyunsaturated fatty acids (PUFA) was selected for nutritional evaluation. Three groups of young male mice were fed AIN93G diet substituted with 0, 5% or 20% dried algae powder, respectively, for 4 weeks. Compared with control diet, feeding 5% algae increased daily weight gain and feed intake. In contrast, 20% algae decreased both parameters and also cholesterol level in serum and triglycerides in the liver. Effects of algae feeding on the lipid content of mouse serum and liver were examined by high-resolution liquid chromatography-mass spectrometry (LC-MS) analysis and multivariate data analysis. Besides altering the fatty acid composition, algae feeding increased the relative abundances of sphingomyelins and PUFA-containing triglycerides while decreased the relative abundances of lyso-phosphatidylcholines in mouse lipidome. Overall, algae feeding is not only an alternative source of fatty acids, but also has regulatory effects on the metabolism of triglycerides and phospholipids.

15. **Nutritional Quality of Snacks Commercialized in Portugal.** T.G. Albuquerque, H.S. Costa, A. Sanches-Silva, and M.B.P.P Oliveira*, University of Porto, Portugal.

Objective: To evaluate nutritional quality of 22 snacks available in Portugal, considering its total fat, salt and fatty acids profile. Moreover, commercial and supermarket brands of each product were compared to assess a possible effect of price on the nutritional quality of foods.

Methods: Twenty two samples of snacks (cream crackers, popcorns, cheese snacks and salty biscuits) were acquired in supermarkets. Total fat determination was performed by acid hydrolysis method followed by Soxhlet extraction with petroleum ether. The salt content was determined by Charpentier Volhard's titration. Fatty acids methyl esters were analyzed using gas chromatography coupled with flame ionization detection.
Results: Snacks have high total fat contents, varying between 3.67 ± 0.0 and 36.7 ± 0.8g/100 g of edible portion. With respect to salt content, the values ranged from 0.0465 ± 0.0 (popcorn) to 4.49 ± 0.1g/100 g (salty biscuit). In our study, the most abundant fatty acids in the analysed samples were palmitic acid (C16:0) and oleic acid (C18:1). Considerable differences in total fat and salt contents were found between commercial and supermarket brands.

Conclusions: This study demonstrated that snacks commercialized in Portugal can greatly contribute for the intake of fat, salt and saturated fatty acids.

16. **Portuguese Autochthonous Acorn Species: Nutritional Analysis.** R. Pacheco, A.S.G. Costa*, A. Vinha, and M.B.P.P. Oliveira, University of Porto, Portugal.

The development of rural areas depends on sustainability policies to promote agricultural and production systems, in order to increase those populations' income. To attain this purpose it is important to find new opportunities to valorize the available, but still underexploited natural resources (1). Herein, the aim of this study was to compare the nutritional profile of kernel, shell and the whole fruit of Quercus faginea, a Portuguese autochthonous acorn species, intending new alternative applications (functional foods).

Samples were collected in Trás-os-montes, north of Portugal. Chemical analyses to determine ash, protein and fat contents were performed according to AOAC methods (2).
Overall, in this study, acorn presents a high content of carbohydrates (>80%) and low fat and protein contents (< 4% and < 8%, respectively). However, some variations were observed in the different fractions of the fruit. Acorn kernel is a good source of carbohydrates (85%) and presents the highest fat content (4%), and shell the lowest fat content (0.5%). The whole fruit presented the highest ash (2%) and protein (8%) contents.

The results of this work highlight this fruit as a promising source for further applications as an added value ingredient.

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17. **Simple Low-temperature Process for Selective Recovery of Vitamin E from Vegetable Oils.** K. Hiromori, N. Shibasaki-Kitakawa, K. Nakashima, and T. Yonemoto, Tohoku University, Japan.

A novel and simple low-temperature process to selectively recover vitamin E (V_EH, tocopherols and tocotrienols) from vegetable oils is proposed. The process consists of three operations: esterification of free fatty acids (FFA) with a cation-exchange resin catalyst, adsorption of V_EH onto an anion-exchange resin, and desorption of V_EH from the resin. In the esterification step, FFA, which are the main components of the raw oils and that competitively adsorb onto the resin, were converted into non-active fatty acid esters with a high conversion of over 98%. In the adsorption step, V_EH and the residual FFA were adsorbed onto the resin. In the desorption step, V_EH were first eluted from the resin and then FFA were also eluted. The degradation of tocopherols did not occur. The recovery ratios were 100% for tocopherols and 70% for tocotrienols and those are higher than those of the conventional multi-stage molecular distillation process. The V_EH-rich fraction obtained did not contain any sterols or triglycerides, commonly found in the product of the conventional process. The purity of V_EH was 81wt% and this is higher than that of the conventional process.

18. **Vitamin E Content of Rainbow Trout Muscle After Feeding a 5% Dietary Supplementation of Seaweed *Gracilaria vermiculophylla*.** M. Araújo, R.C. Alves, F.B. Pimentel, T.J.R Fernandes, A.S.G. Costa*, L.M.P. Valente, and M.B.P.P. Oliveira, University of Porto, Portugal.

Due to its content in bioactive compounds (1), seaweeds have been largely studied as supplement in fish feed, regarding its effects on fish growth (2) and muscle composition (3). However, the impact of dietary seaweed on vitamin E levels of flesh have not been evaluated.

This work studied the effects of dietary inclusion (5%) of seaweed *Gracilaria vermiculophylla* (GRA) on flesh composition of rainbow trout compared to a control diet (CTRL). Moisture and protein were analysed using official methods (4), lipid content was evaluated using Folch procedure (5) and vitamin E was extracted with n-hexane and quantified with normal phase-HPLC with fluorescence detection.

GRA increased flesh moisture (74%) when compared to CTRL group (71%) and a decrease in total lipid content was observed (5.15 and 4.46% for CTRL and GRA, respectively). Muscle protein content did not vary among dietary treatments (20-21% DM). The inclusion of *G. vermiculophylla* in rainbow trout diets resulted in a significant decrease of α-tocopherol content from 18.3 (CTRL group) to 15.1mg/kg of flesh in GRA group.

Despite its low contribution to vitamin E levels in trout flesh, *Gracilaria vermiculophylla* does not affect flesh protein content.

19. **Investigation on the Qualities of Olive Drupes and Olive Oils Produced in China.** Y. Xue, D. Zhang, L. Zhu, and Z. Duan, Academy of State Administration of Grain, China.

Longnan is the most suitable region for olive cultivation in China. The cultivated area achieved to 29220 hectares, among which the fruiting area was 10333 hectares in 2014. The productions of fruits and the virgin olive oils would be 15500 tons and 2325 tons, respectively. In this work, the systematic analysis of the qualities of the olive drupes (including Ezhi 8, Chenggu 32, Picholine, Leccine, Frantoio, Arbequina, Picual and Koroneiki) and the virgin olive oils produced in Longnan was performed for the first time. The maturity index ranged from 2.3 to 6.5. The polar diameters, equatorial diameters and fruit weights were 15.5-27.2mm, 11.1-19mm, and 0.9-5.6g, respectively. The flesh rate and water content of the drupes were 78.1-86.8% and 42.4-63.2%, respectively. The oil content was ranged from 15.1%

to 30.6%. The saturated, monounsaturated and polyunsaturated fatty acid contents were 14.7-18.4%, 63.9-80.8%, and 3.3-20.0%, respectively. The major triacylglycerols were OOO, SLO, LOO, and POL+SLL, of which the contents were 24.7-48.2%, 19.3-27.9%, 6.6-21.0%, 3.1-13.0%, respectively. The contents of α-tocopherol and γ-tocopherol were 126.2-292.8mg/kg and 8.9-42.5mg/kg, respectively, while both β- and δ-tocopherol were less than 10mg/kg. The squalene content was ranged from 2709.7 to 7856.5mg/kg.

20. **Revalorization of Four Discarded Species of the Alboran Sea as PUFA Concentrates Source: Seasonally Characterization of Their Fatty Acids Regiodistribution.** P.J. García-Moreno, R. Morales-Medina, R. Pérez-Gálvez, Muñío, A. Guadix, and E.M. Guadix, University of Granada, Spain.

The regiospecific distribution of fatty acids within the glycerol backbone might determine the further applications of oils. Sources with high content of EPA and DHA in the sn2 location could be considered as raw materials for the production of structured lipids. On the other hand, since the production of PUFA concentrates requires hydrolysis or esterification of triacylglycerol, those oils with high global PUFA content, but not specifically in sn2 position might be considered as an adequate raw material.

The aim of this study was to seasonally characterize the nutraceutical properties of the oil extracted from four discarded fish species in the Alboran Sea, namely axillary seabream (*Pagellus acarne*), sardine (*Sardina pilchardus*), horse mackerel (*Trachurus mediterraneus*) and blue whiting (*Micromesistius poutassou*). To that end, proximate composition, lipidic profile, regiospecific distribution of fatty acids within the glycerol backbone and three nutritional parameters (atherogenicity and thrombogenicity indexes and hypercholesterolaemia-hypocholesterolemic ratio) were analyzed. In all cases healthy values for the nutritional indexes were obtained along the year. PUFA was the majoritarian fraction in the global profile and presented selectivity towards sn2 position. Since the average content of DHA and EPA occupying sn2 position was 38.7±8.1 mol%, these oils are considered as omega-3 concentrates source.

21. **BCFA Content and Positional Distribution in Lipids of Animal Milk Compared with That of Fish Oil.** Y. Yan[1], X. Wang[1], Y. Liu[2], J. Xiang[3], Y. Wang[2], and Q. Jin[1], [1]Jiangnan University, China, [2]Food Inspection Authority of Zhangjiagang Entry-Exit Inspection and Quarantine Bur, China, [3]Wuxi Maternity and Child Health Care Hospital, China.

Branched chain fatty acids (BCFA) are primarily saturated fatty acids with one or more branches (mainly methyl group). As different species have different metabolic pathways, composition and content of BCFA in milk fat may vary from each other correspondingly. However, a thorough investigation and statistical evaluation of BCFA distribution in nature is hardly seen. Thus BCFA content and positional distribution in lipids of animal milk compared with that of fish oil was conducted in this paper. A GC/EI-MS-selected ion monitoring (SIM) method was applied in order to link the unique selectivity of GC/EI-MS with the high precision and sensitivity of the SIM mode. Rumen microorganism and other microorganism inside animal and human body are mostly mesophylls, which have lower amount of iso fatty acid than anteiso fatty acid. As some of the BCFAs in different samples may come from bacteria, to be precisely, these BCFAs are possibly come from the bacterium inside their body not from bacterium in the food they eat. Different species have different distribution of BCFA in the triacylglycerides, which may indicate that mechanism of acylation of fatty acids to glycerol sn-2 position could possibly vary with different species.

22. **Detection of a Major Peanut Allergen in Food Using a Biosensing Approach.** R.C. Alves[1,2], F. Pimentel[1], H. Nouws[2], M. B. González-García[2], M.B.P.P. Oliveira*[1], C. Delerue-Matos[2] [1]University of Porto, [2]Polytechnic Inst. of Porto, Portugal.

Peanut (*Arachis hypogaea)* is one of the most allergenic foods. Ara h 1 (a major allergen) is a seed storage protein, thermostable and resistant to digestion in the human gut, being recognized by serum IgE from more than 90% of peanut-allergic patients (1).

In this work, a gold-nanostructured voltammetric immunosensor for Ara h 1 analysis was developed, validated, and used to detect the allergen in food samples (2).

A gold-nanostructured screen-printed carbon electrode was used to develop a two-monoclonal antibody sandwich-type immunosensor for Ara h 1 analysis. Electrochemical detection was based on

alkaline phosphatase-catalyzed metal precipitation. Optimization of variables involved in the immunosensing strategy was performed, regarding the optimum concentrations of each antibody, the number of the steps to perform analysis, and the time of the assay.

The proposed immunosensor provided precise and accurate results and presented very low limits of detection and quantification (3.8 and 12.6 ng/ml, respectively). It was able to detect very small amounts of Ara h 1 (0.1%) in a complex food matrix (cookies).

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