FROM KRILL TO WHALE: AN OVERVIEW OF MARINE FATTY ACIDS AND LIPID COMPOSITIONS

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The variety of lipid classes and fatty acid compositions from zoo and phytoplankton to marine mammals including all invertebrates (mollusca, cephalopoda, crustaceans, Echiuroidea) and fish will be overviewed. In fact, the composition of this energy resource changes all along the animal food chain and has now a growing interest for human consumption and for the pharmaceutical and nutraceutical industries.

Since prehistoric times, marine oils have been mainly used as an illuminant in lamps and as lubricants. Their medicinal virtues have been pointed out in the middle of the XIXth century. However, it was only with the publication of the Danish epidemiological studies of Bang and Dyerberg in the early 1970s, that the true worth of the research on polyunsaturated fatty acids (PUFAs) has been understood. They postulated that the low incidence of coronary heart disease of Greenland Eskimos might be related to their distinctive dietary habit and use of lipids rich in PUFAs. From this milestone, considerable evidence from epidemiological, clinical and biochemical benefits of n-3 long chain PUFAs, mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids was shown. These PUFAs exert a strong positive influence on human health, preventing the risk of cardiovascular diseases, several immune disorders affecting neural development and neurodegenerative diseases and they protect against tumorigenesis. For these reasons, regular fish consumption to provide approximately 200-500 mg per week of EPA and DHA has been recommended by the different health organizations. Consequently, these studies led to a strong demand of fish oils and fish oil concentrates on supplement and functional food markets. The need is now so important that the natural resource is declining, leading to the development of aquaculture with the consequence that farmed fish being fed with regular diets, their lipid composition is notably different from wild species, which varies all year long. It is well established that the lipid composition of fish muscle is influenced by the diet and also depends on the effects of environmental factors (temperature, oxygen concentration in sea water) and endogenous medium (physiological state and individual variability). As well known, lipid-rich tissues contain triacylglycerols as major lipids, while tissues low in lipid content may be dominated by polar lipids such as phospholipids.

To date, most of marine living organisms have been investigated for their lipid composition. However, few studies on lipids sources such as ceramides, phospholipids, glycolipids, alkylglycerols, etc., which may have potentially many benefits over "simple" triacylglycerols from fish oils are undertaken.

Studies on non-conventional marine sources with high contents of LC-PUFAs, (microalgae, squid, starfish, fish roes, crustaceans…) are now underway, and novel oil extraction processes are used.

MARINE LIPIDS OVERVIEW: MARKETS, REGULATIONS, AND THE VALUE CHAIN

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The market for EPA and DHA omega-3s has grown rapidly in recent years sue to science, industry's willingness to collaborate on quality standards, and investments in consumer education globally. The rapid growth of the EPA and DHA supplement market has led to increased interest in the space, and now it is common to find omega-3s in foods, beverages, pet foods, infant formulas, clinical nutrition and pharmaceuticals. Many of these products have reached blockbuster success levels, and in fact have much lower rates of failure than traditional consumer products. The industry has moved beyond positioning omega-3s solely as a cardiovascular product, and now smaller niches are being developed around brain, maternal, child development, joint, and anti-inflammatory benefits. Widespread consumer awareness of omega-3s, and growing acceptance of its importance in human nutrition, has contributed to new product development in these areas.

It has also led to research into new sources of EPA and DHA through algal and yeast fermentations, genetically modified crops, and non-traditional marine organisms. These sources will continue to become more important. The traditional fish oil market has been designed on delivering oils with the maximum amount of EPA and DHA available naturally. However, limited species of fish are suitable for producing oil to these specifications, and the fisheries of those that do are managed to produce a maximum sustainable yield of fish. The market for EPA and DHA omega-3 oils is growing so rapidly that demand may exceed the legal catch limits within a few years. This will force the industry to explore new sources and solutions.

The market growth will lead specific sectors of the omega-3 value chain to make some difficult decisions. Crude oil manufacturers may seek to move down the value chain and producing finished oils. Refiners may continue to invest strategically in securing sources of crude oil supply. Concentrators may produce low concentrates from crude fish oils with lower concentrations of EPA and DHA. Various sectors of the finished goods market will have different price sensitivities that may force specific companies to invest in new technologies to help them use non-traditional oils.

SEAFOOD MARKET IN FRANCE

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Seafood consumption in France has increased from 25 to 35 kg per capita in 18 years (1990-2008). As French landings have decreased (quota and fleet reduction) and aquaculture has been stable for the past ten years, the growth of consumption was fuelled by imports (2.2 million tons (live weight) in 2008). Meanwhile, exports have remained stable for 10 years

France's main supplier for France is Norway, with 124 000 tons imported in 2008 (live weight) including 84 000 tonnes of salmon. About 40 % of imported volume comes from the European Union (UK, Spain, and the Netherlands) and 10 % from the EFTA countries (Norway, Iceland, Switzerland).
Concerning exports, the main markets for French seafood products are Spain and Italy, and the EU represents 75 % of the French export market. France exports processed products (frozen filets, smoked salmon, canned tuna) and high value fresh fish to more lucrative markets in southern Europe.

Looking at household purchases, the increase in consumption mostly benefited chilled delicatessen and frozen products at the expense of fresh seafood. The most popular products are smoked salmon, surimi and chilled, cooked shrimp. Although fresh fish consumption is falling, fresh salmon purchases are still rising, and France is the main European market for Irish and Norwegian salmon farmers.

Therefore, the part of aquaculture in French consumer purchases is growing. In 2008, 27% of the seafood supply came from aquaculture, due to the importance of farmed shrimp, molluscs and fish. Over the last ten years, about 14% of fish supply is made up of farmed products, mostly salmon, but also farmed marine fish (seabass, seabream) and freshwater farmed fish (tilapia, pangasius).

These products, and especially salmon and shrimp are more and more consumed fresh, chilled or frozen but also incorporated into chilled or frozen deli products.
World aquaculture should increase over the next years, to balance the drop in fishery landings and fulfil growing demand. With the development of global aquaculture likely to continue and the French reliance on imports, the portion of farmed products in overall French seafood supply should increase.

LIPIDS OF FARMED FISH: MAIN FACTORS AFFECTING CONTENT AND COMPOSITION

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Aquaculture has expanded rapidly to fulfill the increased demand for fish. Indeed, fish represent a rich source of the n-3 polyunsaturated fatty acids (PUFA), particularly the highly unsaturated fatty acids (HUFA) eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids for the human consumer. Although EPA and DHA are more abundant in membrane phospholipids than in triacylglycerols, the content in EPA and DHA generally increases with the lipid content of fish muscle. There are huge differences in muscle lipid content among fish species resulting in great difference in EPA and DHA content. Some species such as sole and cod, are classified as "lean" species because of a limited capacity to store fat in the muscle (less than 2 g/100 g), some others such as salmon contains more than 12 g/100 g and are classified as "fatty" species. The lipid content of fish flesh varies not only among species, but also within species among individuals depending on age, stage of sexual maturity and also location of fat depots in the muscle. In "fatty" fish, such as salmonids, muscle lipid content increases with age and/or fish size. Fish from farming can also be tailored through selective breeding, feeding and rearing practices. Muscle fatty acid profile generally reflects the fatty acid composition of the feed. Feeding fish with ingredients of marine origin results in fish flesh high in EPA and DHA. However, the feed-grade fisheries that have supplied the raw materials for aquaculture feeds have reached sustainable limits. Consequently, the need to develop feeds based on sustainable alternatives to fish oil to produce healthy fish that remain highly nutritious has increased. Fish oil can be replaced by a blend of vegetable oils in aquaculture feeds during a large part of the life cycle. The resulting reduction of EPA and DHA content in fish flesh can be overcome with a finishing diet high in fish oil, some months before slaughter. Another strategy that is currently investigated aims to maximize the deposition of EPA and DHA in the edible part of farmed fish through selective breeding. EPA and DHA are highly susceptible to attack by oxygen and other organic radicals and resultant damage to PUFA can have serious consequences with potential pathological effects on cells and tissues. So a special attention should be paid to ensure an efficient physiological antioxidant protection of farmed fish that involves both endogenous components, such as free-radical scavenging enzymes, and exogenous dietary micronutrients including vitamin E and selenium.

THE EU HEALTH CLAIMS REGULATION : IMPACT ON THE MARINE LIPIDS

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European Regulation (EC) No. 1924/2006 applies to all types of pre-packed food for the final consumer, including food intended to supply hospitals, canteens and similar mass caterers, bearing nutrition and health claims.

Commercial communications (*labelling, presentation or advertising of foods*), trade names and other brand names which may be construed as nutrition or health claims are covered by the Regulation.

Since the date it was brought into force (1 July 2007), all nutrition and health claims for food products must be authorised prior to the marketing of the products, either by means of a nominative evaluation procedure or a generic evaluation.

In light of the provisions of the new Regulation and the transitional measures in effect, what is the future of lipid "health communication" and more particularly of marine lipid communication? For certain lipids of marine origin (*e.g. EPA/DHA, etc.*) play an unquestionable nutritional – not to say health – role in the human diet, a fact which is widely accepted by the scientific community

OVERVIEW OF CURRENT AND FUTURE EUROPEAN REGULATIONS ON MARINE LIPIDS ISSUES

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Nutritionist

This presentation will give a short overview about current and future European regulations concerning the issue of unsaturated fatty acids in fishoils, especially omega-3 fatty acids (EPA, DHA). Several studies have provided evidence that the intake of omega-3 fatty acids shows a positive effect on a series of health issues.

These legislative proposals as well as national and European recommendations are of interest:

The main legislative dossier is the Health Claims Regulation (1924/2006/EC). The Annex, Article 13.1 and 14 of this regulation establishes the background of allowed health claims.
The latest developments of the revision of the Annex of this regulation (source of omega-3 fatty acids; high of omega-3 fatty acids) and the discussion of main lipid scientists shows that it is necessary to defend marine lipids intake for the health benefit of the consumers.

Th Eu Health Claims Regulation will establish specific Nutrient Profiles (Article 4 of 1924/2006). This dossier is still under discussion and can have an important impact on the fat and fatty acid discussion.

In this context the Commission proposal of the Food Information Package (FIP), which is also still in the codecision procedure of European Parliament and Council has the purpose of simplifying the current food labelling directive. The aspect of nutrition labelling (GDA) can have an impact for the discussion on marine lipid issues.

A review of EFSA opinions (European Food Safety Agency) in this context provides some insights in the latest developments in the area of dietary reference intakes for fat and fatty acids.

FROM COD LIVER OIL TO HIGH CONCENTRATED FISH OIL RICH IN PUFA: OVERVIEW OF FISH OIL PROCESS

Anne-Gaëlle GROSDEMANGE

POLARIS

Overview of the most common extraction methods of fish oils from cod liver oil to high concentrated fish oil rich in PUFA.

Traditionally fish oil industry was associated to fish meal industry and the processing methods were mostly adapted to the raw material form: whole fish or part of fish (liver, head, by-products). Now, the processing line are more and more technically advanced when the customer requests for deodorised, uncontaminated and rich in PUFA oils increase.

New process of extraction as cold press extraction emerges coming from vegetable oil industry. Refining including Deodorization and Decontamination is completed by stripping and molecular distillation decreasing the level of contaminants close to the detection limit of analytical methods.

The lipochemistry allows to concentrate the natural content of Omega 3: esterification then concentration by molecular distillation or CO2 supercritical plus transesterification (chemical/enzymatic) for TG form.

Possibility of fish oil process is "unlimited" to offer to the market the benefice of Omega 3 with safe, traceable, tasty fish oils.

PROCEDES D'OBTENTION D'ACIDE GRAS EX MICROALGUES POUR LA COSMETIQUE. QUELLES EVOLUTIONS POUR DEMAIN ?

Alexis RANNOU

SOLIANCE

REMOVAL OF ENVIRONMENTAL CONTAMINANTS FROM FISH OILS

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Crude fish oil is an important ingredient in fish feed for fish farming; it can be used as neutraceutical (cod liver oil, EPA/DHA concentrates) or incorporated in healthy foods after refining.

Major components of fish oil have positive nutritional properties. Polyunsaturated fatty acids can go up to 40% with variable proportions of EPA (C20:5) and DHA (C22-6) [omega-3 fatty acids]. Content of fat soluble vitamins is usually high in combination with low caloric value. Unfortunately, fish oil is also sensitive to degradation products (polymeric and oxidized fat, increased polar content, off-flavor problems …). The beneficial nutritional properties of fish oil are in great contrast with the presence of heavy metals and persistent organic pollutants (POP's) such as pesticides, polychlorobifenyls (PCB's), hexachlorobenzene and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F's), mainly originating from the environment. These molecules accumulate in fat adipose tissues and end up in the extracted fat.

Usual steps for fish oil refining are neutralization, bleaching (including a detoxification step), winterization (optional) and deodorization. Processing difficulties consist in finding the best operating conditions for an effective removal of unwanted contaminants and a maximal preservation of the nutritional qualities (processing duality).

Different adsorbents (bleaching earth, filter aid, silica powder and active carbon) were tested for the best removal of PCB's and PCDD/F's; it was shown that bleaching earth, filter aid and silica have no significant effect on detoxification while removal of > 90% non-ortho PCB's and > 99% PCDD/F's was possible with active carbon. Unfortunately, mono-ortho PCB's were significantly less absorbed by active carbon.

Active carbon treatment was optimized based on a response surface model for total decontamination to confirm the best process conditions.

Deodorization was further investigated. Processing temperature had a very pronounced effect on the reduction of PCB's and PCDD/F's. Already at 210°C, all of them were efficiently removed to a level below the limit set by European Legislation. Temperature increase was limited by the risk of degradation of EPA/DHA.

Combination of active carbon treatment and deodorization was shown to be the best compromise to remove majority of the contaminants and to preserve the nutritional quality of fish oil. At low active carbon dosage and deodorization temperature below 200°C, the total contamination level of PCB's and PCDD/F's can be reduced below level imposed by European Legislation.

LIPIDES MARINS ISSUS DE LA VALORISATION DES CO-PRODUITS DE LA PECHERIE BRETONNE

Patrick ALLAUME

ID.MER

BIOTEChnological exploitation of MARine products and by-products: the BIOTECMAR project

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In the Atlantic area, a large number of direct and indirect economic activities are generated by the exploitation of marine resources. These natural resources can represent real assets if they are exploited under stringent environmental conditions. Simultaneously, fishing, aquaculturing and seaweed harvesting do generate a large amount of by-products. These under-utilized biomasses can represent valuable starting materials for the production of active substances and ingredients for human alimentation and animal feeding, nutrition, cosmetics, well-being, etc.

BIOTECMAR is a Transnational project (INTERREG IVB Atlantic Area) whose objective is to implement the networking of competences and know-how in the domain of biotechnological exploitation and valuation of marine living resources, and to sustain the development of an integrated value chain, gathering biotechnology laboratories, transfer centers as well as all the stakeholders of the production chain: collectors and producers of raw material, transformers, ingredients and active compounds manufacturers.

The various sectors concerned by the project are the following:

- The fisheries, aquaculture, seaweed harvesting and seafood processing as source of raw materials
- The fish waste conservation, collection, transport and processing
- The production and commercialization of bioactive compounds and/or ingredients derived from processing to be used for the food, feed, nutrients, cosmetics and therapeutic industries
- The development and transfer of R&D in marine biotechnology.
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A continuous chain approach is being considered, embracing the total seafood production chain system from stocks to market, in order to properly address the main objective and to detect complementarities and stimulate synergies from processing to commercialization.

Actions will be undertaken through 6 main activities, which will allow to:

- Reinforce the cooperation between research centers/laboratories, technical centers and SMEs in the field of biotechnological valuation of marine resources, through network actions implemented within the project (for more information, see www.biotecmar.eu)
- Bring off dissemination actions and technology transfers towards SMEs about sustainable and added-valuation of underexploited marine resources,
- Organize cooperation between research centers/laboratories and SMEs from Atlantic Area,
- Support the development of new industrial activities related to optimized valuation of natural marine resources of Atlantic regions,
- Disseminate project's results.

The project is gathering 12 partners from Spain, France, Portugal and Ireland. In each participating country, the partnership includes research/R&D organisms and technical transfer centers, in order to insure proper dissemination to SMEs. The various competencies and fields of activities of partners are fully complementary and representative from the marine resources value chain.

The first part of the talk is dedicated to the presentation of BIOTECMAR project. In the second part, examples of marine, commercially available functional foods or nutraceutical ingredients carrying bioactive properties will be presented in order to demonstrate the interest of biotechnological exploitation of marine resources.

LIPIDES MARINS ET MALADIES CHRONIQUES : L'EXEMPLE DES MALADIES CARDIOVASCULAIRES

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Linoleic acid (LA) and alpha-linolenic acid (ALA) are precursors of omega-6 and omega-3 fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized from ALA. These fatty acids play important role in various biological functions. The effects of omega-3 on cardiovascular risk factors - such as lipoprotein levels, blood pressure and glycemia - are very modest at standard low doses, significant only for large intakes. Randomized trials have shown that oils enriched in EPA and DHA have an undeniable impact on heart rhythm. Consumers of long-chain fatty acid have lower heart rates than controls. Conversely, patients with established arrhythmias do not appear to benefit from treatment with these fatty acids.

The observation of food habits in population cohorts helped to defined the effects of rich in ALA, EPA, DHA on the risk of myocardial infarction, coronary heart disease and sudden death. For ALA the results are inconsistent and appropriate randomized clinical trials are still conclude. The effects of EPA and DHA are better established. The meta-analysis of cohort studies shows that consumption of omega-3 long-chain (EPA-DHA) reduces total mortality, death from cardiac causes, sudden death and possibly stroke. The data from prevention trials with omega-3 long-chain showed a decrease in coronary events, including fatal, in secondary prevention. These results are relatively consistent to believe that EPA and DHA intake reduces the fatal complications of myocardial infarction. Clinical trials in primary prevention are still missing. However, the sum of evidence support the recommendation for consumption of fish, EPA and DHA in patients at high risk of coronary event.

ANTI-TUMOUR AND ANTI-METASTASIS ACTIVITIES OF NATURAL ALKYLGLYCEROLS : STRUCTURE/ACTIVITY RELATIONSHIP.

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Alkylglycerols (alkyl-Gro) are ether lipids abundant in shark liver oil (SLO), and oral SLO or alkyl-Gro mix from this source have several *in vivo* biological activities including stimulation of haematopoiesis an immunological defences, or anti-tumour and anti-metastasis activities *in vivo*. Composition of natural alkyl-Gro mix contains several alkyl-Gro varying by chain length and unsaturation, and individual anti-tumour activites of each molecule present in natural mix remained unknown. We synthesized six prominent constituents of natural alkyl-Gro mix, namely 12:0, 14:0 16:0, 18:0, 16:1 n-7, and 18:1 n-9 alkyl-Gro. Using an *in vivo* model of grafted tumour in mice (3LL cells), we studied and compared the oral anti-tumour and anti-metastasis activities of each of these 6 alkyl-Gro. 16:1 and 18:1 alkyl-Gro showed strong activity in reducing lung metastasis number, while saturated alkyl-Gro had weaker (16:0) or no (12:0, 14:0, 18:0) effect. Spleen weights at day 20 after graft were also measured and showed tremendous variations depending on the treatment. Tumour graft resulted in a raise in spleen weight in control group, this raise was nearly abolished in 16:1 and 18:1 alkyl-Gro- treated mice, and was reduced in 14:0 and 16:0 alkyl-Gro-treated mice. Conversely, 18:0 alkyl-Gro treated mice showed spleen weigh raise as compared with untreated grafted mice.

In order to evaluate anti-angiogenic activities of these different alkyl-Gro, we also studied their *in vitro* inhibiting effect on VEGF-stimulated human umbilical vein endothelial cell (HUVEC) migration in Boyden chambers. At 20 µM, a non cytotoxic concentration, the order of potency for migration inhibition was 18:1 > 16:0 > 16:1 > 12:0 > 18:0 >14:0.

These new data demonstrate a prominent role of unsaturation in the anti-tumour and anti-metastatic activities of alkyl-Gro.

UNCOMMON FATTY ACIDS FROM MARINE ORGANISMS: OCCURRENCE, IDENTIFICATION, BIOSYNTHESIS AND BIOLOGICAL ACTIVITIES

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In addition to the common polyunsaturated fatty acids (FA) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids of major interest in health and nutrition, marine organisms, in particular invertebrates and algae, are able to produce unique FA including branched, hydroxylated, methoxylated, halogenated, acetylenic and non-methylene-interrupted structures. This short overview focuses of the principal classes of FA possessing uncommon structures, especially those of increasing interest regarding their promising biological activities. The biosynthetic pathways, and the structural and functional roles of these uncommon FA are often not yet known.

The most encountered non-methylene-interrupted (NMI) FA are undoubtly 22:2D7,13 and 22:2D7,15 acids and their precursors occurring mainly in mollusks, and a series of D5,9 FA occurring mainly in sponges. It has been suggested for them a role in a higher resistance to oxydative processes in biological membranes. Recently, intense research effort has been conducted to investigate the biomedical potential of these unusual FA, in particular branched monoenoic, acetylenic, methoxylated and NMI FA. Acetylenic FA found in sponges revealed an activity against bacteria and fungi. Methoxylated FA seem limited to primitive organisms such as cyanobacteria, bacteria and sponges and showed an interesting antimycobacterial effect. The D5,9 FA displayed important antiprotozoal activity, in particular antiplasmodial activity, represent the most promising FA topoisomerase-I inhibitors to date and displayed a toxicity towards cancer cell lines.

LES ACIDES GRAS NON CONVENTIONNELS DES ORGANISMES MARINS: OCCURRENCE, IDENTIFICATION, BIOSYNTHESE, ACTIVITE BIOLOGIQUE

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En plus des acides gras (AG) poly-insaturés conventionnels - par ex. eicosapentaènoïque (EPA) et docosahexaènoïque (DHA) - d'intérêt majeur en santé et nutrition, les organismes marins, en particulier invertébrés et algues, produisent des types exceptionnels d'AG incluant des acides ramifiés, hydroxylés, méthoxylés, acétyléniques et insaturés non maloniques. Ce rapide survol se focalisera sur les principales classes d'AG à structures rares, tout particulièrement ceux qui suscitent un intérêt croissant en raison de propriétés biologiques prometteuses. Les voies de biosynthèse, les rôles structuraux et fonctionnels de ces AG restent souvent mal connus.

Les plus fréquemment rencontrés des AG non maloniques sont les acides 22:2D7,13 et 22:2D7,15 surtout présents chez les mollusques, et les AG D5,9 surtout chez les éponges. Un rôle dans la résistance des membranes aux phénomènes d'oxydation a été proposé pour ces AG. Les AG acétyléniques trouvés chez les éponges ont montré une activité significative contre bactéries et micromycètes. Les AG méthoxylés, limités aux organismes primitifs tels que cyanobactéries, bactéries et éponges, possèdent une activité antimycobactérienne intéressante. Les AG D5,9 ont révélé une activité importante contre les protozoaires (en particulier activité antiplasmodiale), représentent à ce jour les plus prometteurs des AG inhibiteurs de topoisomérase-I et ont une toxicité vis-à-vis de lignées cellulaires cancéreuses.

NEPTUNE KRILL OIL: SUPERIOR MARINE OMEGA-3 PHOSPHOLIPID

Wael MASSRIEH

NEPTUNE

CHEVREUL MEDAL

CHALLENGES WHEN DEVELOPING OMEGA-3 ENRICHED FOODS

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There is an increasing interest in developing functional foods containing marine omega-3 fatty acids. However, such foods are very susceptible to lipid oxidation, which will give rise to undesirable off-flavours and unhealthy oxidation products. Hence, efficient strategies to prevent lipid oxidation are required. Unfortunately, lipid oxidation and antioxidant mechanisms in multi-phase food systems are very complex and many factors can influence the rate of lipid oxidation and the efficacy of different antioxidants in each food system. This includes different factors such as pH, type of emulsifier, interactions between ingredients, presence of metal ions, processing conditions etc. Because of this complexity it is difficult to predict the behaviour and efficacy of antioxidants in multi-phase systems including real food emulsions such as milk, salad dressings, and mayonnaise. This presentation will discuss different strategies to prevent lipid oxidation in fish oil enriched foods including optimisation of processing conditions and oil quality, choice of emulsifier and antioxidants. As for the addition of antioxidants the efficacy of different antioxidant principles and mechanisms in omega-3 enriched food emulsions will be summarised. This includes discussion of the efficacy of different antioxidants such as caffeic acid, ascorbyl palmitate, tocopherol, propyl gallate and EDTA in different omega-3 enriched foods.

AFECG THESIS PRICE

GASTROINTESTINAL LIPOLYSIS OF LIPID-BASED EXCIPIENTS INTENDED FOR THE ORAL DRUG DELIVERY OF POORLY WATER-SOLUBLE DRUGS

Sylvie FERNANDEZ

Oral administration of drugs exhibiting poor solubility in gastrointestinal fluids is one of the main challenges of the pharmaceutical industry. To improve oral bioavailability of poorly water-soluble drugs, innovative formulations are developed such as lipid-based formulations which form, under gentle stirring, oil-in-water emulsions or microemulsions.

Among the different lipid-based excipients available so far, Labrasol® and Gelucire® 44/14 (mixture of acylglycerols, PEG esters, and free PEG) are able to form microemulsions in contact with gastrointestinal fluids. Although Labrasol® and Gelucire® 44/14 significantly increase the bioavailability of various poorly water-soluble drugs, the mechanisms underlining these effects still remain to be elucidated.

Digestive lipases are soluble enzymes, able to hydrolyze ester bonds of insoluble substrates such as long chain triacylglycerols but also various ester bonds of soluble or partially soluble substrates. Acylglycerols are known to be good substrates of digestive lipases and we showed that PEG esters were also substrates of digestive lipases. Since Labrasol® and Gelucire® 44/14 contain various esters, that might be hydrolyzed in the gastrointestinal tract, their lipolysis might have a major influence on the fate of a poorly water-soluble drug in the gut.

In humans, the digestion of dietary triacylglycerols starts in the stomach with the gastric lipase. and continues in the small intestine where the chyme is mixed with pancreatic lipases and bile.

To understand the ability of Labrasol® and Gelucire® 44/14 to increase the bioavailability of poorly water-soluble drugs *in vivo*, we developed an *in vitro* method to evaluate the *in vivo* gastrointestinal lipolysis of these excipients. This method takes in consideration both gastric and intestinal steps of lipolysis whereas in most of the studies on bioavailability and/or solubility of poorly water-soluble drugs, gastric lipolysis is never taken in consideration. Experimental conditions were adapted from *in vivo* data recorded at 50% of gastric emptying of test meals both in the stomach and in the duodenum. Enzymatic solutions were prepared according to *in vivo* secretions of lipases during a meal and pH values used for both gastric and intestinal lipolysis steps were in agreement with the average pH values found in the stomach and in the duodenum at 50% of gastric emptying. A dilution was also performed at the end of the gastric lipolysis step to reflect the dilution of the chyme by pancreatic and biliary secretions.

Using this model, we studied the changes in the composition of both Labrasol® and Gelucire® 44/14 during their *in vitro* gastrointestinal lipolysis. It was shown that the gastric lipolysis step significantly modified the composition of both excipients. This finding highlights the importance of gastric lipolysis *in vivo* which could have a significant impact on drug solubilization in gastrointestinal fluids.

We also performed the *in vitro* gastrointestinal lipolysis of Labrasol® and Gelucire® 44/14 containing a poorly water-soluble drug, cinnarizine, so as to evaluate the impact of excipients' lipolysis on cinnarizine precipitation. It seems that the lipolysis of Labrasol® was a prerequisite to prevent cinarizinne from precipitation and to maintain it in an aqueous solution whereas, when cinnarizine was formulated with Gelucire® 44/14, the lipolysis of Gelucire® 44/14 did not affect the amount of drug dissolved in an aqueous solution.

FROM SHARK SQUALENE TO VEGETABLE SQUALENE. WHAT DEVELOPMENTS?

Jacques MARGNAT

SOPHIM

Squalene ($C_{30}H_{50}$) is a versatile biomolecula in the vegetable and animal kingdom. It is widely used in cosmetic industry in its hydrogenated form ($C_{30}H_{62}$). Being one of the main component of the human sebum (12 to 15%), it plays a main role in the stratum corneum and the skin moisturization. Used since 1997 in the vaccine adjuvant composition, this use has been accelerated in 2009 with the H1N1 flu pandemic. Indeed, "Squalene plays a main role on the main factors initiating the immune answer" according to GSK laboratory.

Squalene is usually extracted from deep sea shark liver oil. As the deep sea species reproduce more slowly and that the resources are threatened, Europe has imposed drastic fishing quotas since 2006 in order to preserve those species.

Since 15 years, the fatty acid residues coming from the olive oil refining have been an alternative raw material source, as they contain a squalene concentration. But the recent crisis has shown that olive origin raw materials are insufficient for replacing shark squalene. This crisis took place in 2007 and 2008 and was caused by the speculation of Japanese producers when buying shark liver oil.

So, Sophim has worked since several years on the squalene extraction from new vegetable sources, different from olive. All the oils contain a small percentage of insaponifiable matter whose main components are Sterols, Vitamine E, and Squalene in lower proportion, except in olive oil. The "insaponifiables" are concentrated in the fatty acid distillate residue obtained from the physical refining of oils (steam stripping under vacuum).

We have proved that squalene, being more volatile than sterol and vitamin E, is concentrated as far as 20 times in comparison with its proportion in the original oil. Our R & D has obtained a patent registration n°0854595 in July 7, 2008 for the global extraction of sterols, vitamin E and squalene from vegetable oil refining residues.

This new approach guarantees squalene security of supply, in sufficient quantities, for the future, with cheaper raw materials. As main by-product of the process, it is obtained a biodiesel of second generation. But it will be necessary to make investments for new production capacities in order to reach this target.

OMEGA 3 DANS LES PETITS POISSONS PELAGIQUES DU MAROC : MAQUEREAU ET SARDINE DU SUD ATLANTIQUE

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Grâce à leurs innombrables promesse santé, les poissons gras ont suscité récemment l'intérêt grandissant des chercheurs de part leur contenu nutritionnel en lipides plus particulièrement en oméga 3. Le rôle bénéfique de ces acides micronutriments n'est plus à démontrer dans la protection contre les maladies cardiovasculaires et dans le développement du cerveau.

Cependant, d'une zone géographique à aune autre et au cours de l'année, ces poissons, plus particulièrement les petits pélagiques peuvent être assujettis à des changements environnementaux et à des fluctuations relatives à la disponibilité et la composition de leur nourriture, ce qui pourrait affecter considérablement leur composition en oméga 3.

C'est sur cette toile de fond d'arguments diététiques et partant du fait que jusqu'à maintenant, très peu d'informations scientifiques sont disponibles quant au statut nutritionnel des oméga 3 des petits poissons pélagique au Maroc, le CSVTPM de l'Institut National de Recherches Halieutique d'Agadir avec le concours de l'Institut Russe de Recherche Halieutiques VNIRO s'est attaché au cours de ce travail à mettre la lumière sur les effets de saison et de zone géographique sur ces acides nutriments et ce dans la sardine et le maquereau capturés au sud atlantique.

Mots clés : omega 3, maquereau, sardine, saison, zone géographique, sud atlantique, Maroc

OMEGA 3 IN THE SMALL PELAGIC FISH OF MOROCCO: MACKEREL AND SARDINES OF THE SOUTH OF ATLANTIC SEA

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Through their health benefits, oily fish have recently attracted increasing interest from researchers due to their nutritional content in fat especially omega 3. The beneficial role of these polyinsaturated fatty acids is well established in brain development and in protection against cardiovascular diseases.

However, from a geographical area to another and during the year, these fish, particularly small pelagic may be subject to environmental changes and fluctuations of their food availability and composition, which could significantly affects the composition of Omega 3 in their edible tissues.

Considering this background of diet arguments and considering the fact that until now, very few scientific informations is available regarding the nutritional status of Omega 3 from small pelagic fish in Morocco, the CSVTPM of INRH in Agadir with the assistance of the Russian Institute of Fisheries Research VNIRO focused in this work to clarify the effects of season and geographical area on these polyunsaturated acids in the sardine and mackerel caught from the South of Atlantic sea of Morocco.

Keywords: omega 3, mackerel, sardine, season, geographic area, south of Atlantic sea, Morocco

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APPLICATIONS COSMETIQUES D'EXTRAITS LIPIDIQUES D'ORIGINE MARINE : QUELQUES EXEMPLES

Pierre-Yves MORVAN

CODIF INTERNATIONAL

Abstract :

Sea plants are a vey interesting source of active biomolecules that can improve some skin damages or deregulation linked to dehydration, ageing, sun exposure. This presentation is not an exhaustive overview of all these biomolecules, but more a description of some examples, with different plants collected and/or cultivated in there marine biotopes, according to the respect of environment and sustainable development. In all these examples, the extraction of oily molecules is mainly made using CO2 supercritical fluid extraction. The extracted molecules belong to different lipids family, like phytosterols, triterpenic alcohols, polyunsaturated fatty acids. The presentation will insist on plant cultivation, extracted lipidic molecules, and cosmetic benefits in domain like hydration, anti-wrinkles, slimming or lips pumpling.

Résumé:

Les plantes marines constituent une très intéressante source de molécules à activité biologique pouvant avoir des effets favorables sur les dommages de la peau consécutifs à une déshydratation, un vieillissement ou une exposition au soleil. Cette présentation ne constitue pas une liste exhaustive de ces molécules, mais plutôt une description détaillée de quelques exemples de plantes prélevées ou cultivées dans le biotope marin, en respectant l'environnement et le développement durable. Dans ces exemples, l'extraction des lipides marins a été réalisée par l'extraction au C02 supercritique. Les lipides extraits appartiennent à des familles aussi diverses que les phytostérols, les alcools triterpeniques et les acides gras polyinsaturés. La présentation insistera sur la méthode de culture des plantes, les molécules extraites et les bénéfices cosmétiques dans des domaines aussi divers que l'hydratation, l'anti-rides, les effets amincissant ou repulpant des lèvres.

MICROALGAE: CHALLENGES AND OPPORTUNITIES FOR THE INDUSTRIAL PRODUCTION OF FUELS AND CHEMICALS

Luc HASPESLAGH

TOTAL S.A

The presentation will review in a comprehensive way the current status of micro-algae technology for the production of fuels and chemicals. It will be compared with agriculture of terrestrial energy crops, it will analyze and discuss current state of development from a technology and an economic point of view and review potential business models. It will point out the major challenges to be addressed and how these challenges translate into R&D objectives. It will also clarify the position of TOTAL in relation to this technology.

MARINES LECITHINES : INTEREST IN VECTORISATION OF BIOFUNCTIONAL MOLECULES

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As already known, natural marine lecithin extracts contains high polyunsaturated n-3 fatty acids (PUFAs) among the most famous ones: eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). PUFAs are associated with a variety of health benefits like reducing the risk of coronary heart disease and inflammation.

However, PUFAs show poor bioavailability because of their liability in the gastrointestinal tract. In order to increase their bioavailability, PUFAs must be presented into organized structures like liposomes, micelles or emulsions. Phospholipids are common stabilizers in the three structures; they are also carriers of n-3 long chain fatty acids which are essentially esterified in the sn-2 position.

On the other hand, lots of investigation tends to encapsulate liposoluble bioactive molecules such as coenzyme Q10, chlorins and porphyrins or hydrosoluble ones like glutathione in liposomes, micelles and emulsions.

In this study, we used emulsion type nanostructures, firstly to increase bioavailability of endogenous coenzyme Q10 and glutathione because of their powerful antioxidant effect and their important biological role in all human cells. Secondly the mTHPC were investigated as a model of photosensitizer to reduce cancer and malignant tissues used in photodynamic therapy.

Several methods were used to prepare emulsions like high pressure homogenization, sonication, French press and extruder. The efficiency of emulsions as a vector of bioactive molecules was tested *in vitro* and *in vivo* models.