ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/259175813

Book Chapter: "Fish Oil: Production, Consumption and Health Benefits", Editors: Matthias Van Dijk and Jürgen Vitek, Nutrition and Diet Research Progress Series, Nova Biomedical-Nov...

Chapter · February 2012

CITATIONS
0

READS
189

7 authors, including:

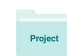
Narcisa Maria Bandarra
Instituto Português do Mar e da Atmosfera
165 PUBLICATIONS **5,197** CITATIONS
SEE PROFILE

Irineu Batista
Instituto Português do Mar e da Atmosfera
86 PUBLICATIONS **2,471** CITATIONS
SEE PROFILE

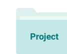
Paulo F. F. Bispo
Universidade NOVA de Lisboa
33 PUBLICATIONS **50** CITATIONS
SEE PROFILE

Maria Leonor Nunes
Instituto Nacional de Investigação Agrária e Veterinária (INIAV)
62 PUBLICATIONS **3,087** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:



RIOVEGEST - Efectos de los cambios en la diversidad e identidad de especies vegetales sobre ríos de cabecera mediterráneos: una aproximación desde los ácidos grasos y carotenoides como trazadores View project



Development of functional ingredients containing omega-3 fatty acids from by-products of the fish industry View project

All content following this page was uploaded by Paulo F. F. Bispo on 27 November 2022.

The user has requested enhancement of the downloaded file.

FISH OIL: PRODUCTION, CONSUMPTION AND HEALTH BENEFITS

Narcisa Maria Bandarra[1*], Irineu Batista[1], Paulo Bispo[1], Maria Leonor Nunes[1], Elena Venegas-Venegas[2], Miguel Ángel Rincón-Cervera[2] and José Luis Guil-Guerrero[2]

[1] INRB/IPIMAR Av. Brasília 1449-006 Lisboa Portugal

[2] Universidad de Almería 4120 Almería Spain

(*narcisa@ipimar.pt)

1. Fish oil production

The annual production of fish body oil presented important fluctuations over the last two decades (Fig. 1). These fluctuations, as mentioned by Barlow [1], have been due to El Niño phenomenon, which has greatly affected the fish oil production in Chile and Peru. This is particularly evident in 1998 where the total production of Peru and Chile only reached 130 thousand tons, which was 15 % of total annual production whereas it generally represented more than 40 %. The El Niño of 1991-1992 and 2002-2003 also caused reductions of fish oils production but they were not as strong as that of 1997-1998.

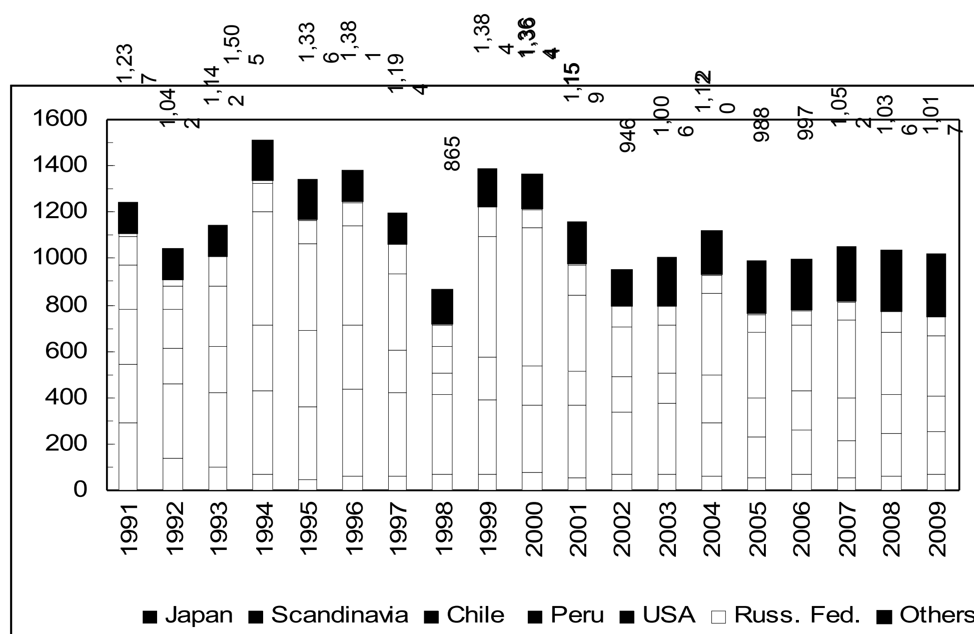


Figure 1 - Major worldwide producers of fish body oil between 1991 and 2009.

The changing uses of fish oil between 1970 and 2009 are shown in figure 2. The most noticeable is the current importance of fish oil in the production of aquafeeds which is

estimated as 81 % of total fish oil production [2]. Only 6 % of fish oil is hydrogenated or has an industrial use whereas in 1970 it was totally used by these two types of oil processing. Another significant aspect is the increasing production of refined edible oil, which is related to the recognized health benefits associated with the fish oil consumption. The percentage of

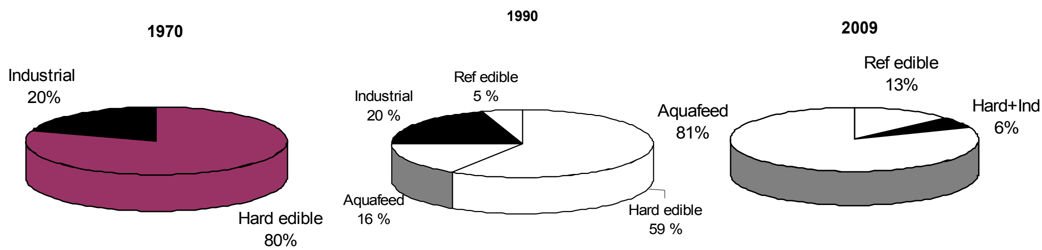


Figure 2 - Evolution of fish oil usage in the period between 1970 and 2009.

fish oil used in aquaculture has substantially increased but between 2000 and 2008 the amount to produce aquafeeds has been stationary despite the continuous growth of global aquaculture production [2]. In figure 3 is shown the percentage of of fish oil usage in aquaculture in 2009. The highest percentage went to salmon and trout production (68 %) but in 2000 it represented 40 % of total [3]. This increase reflects the growing production of salmonids in Norway, Chile, Canada and various European countries.

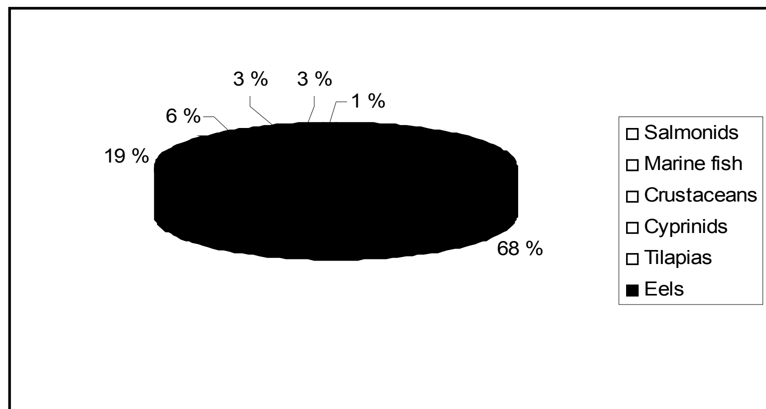


Figure 3

Figure 3 – Percentage of fish oil used in aquaculture in 2009.

1.1 Fish oil recovery

Fish body oil is almost exclusively produced from small pelagic species, such as anchovy, sardine and jack mackerel in South America and sandeel, capelin, sprat, herring and horse mackerel in Europe [3]. In these species the lipids are deposited under the skin, distributed in their muscle tissue and also located in the tissues of the intestine and mesentery. The oil may be also produced from the liver of white lean fish and blubber of marine mammals [4].

Fish oil composition varies markedly depending on a variety of factors including species, sex and season. As reported [5], commercial fish oil is usually composed of over 90

% triacylglycerols (TAGs), about 8 % of mono-, and di-acylglycerols and other lipids such as phospholipids. The unsaponifiable fraction accounts for the remaining 2 % and includes sterols (mainly cholesterol), glyceryl ethers, hydrocarbons, fatty alcohols and fat-soluble vitamins (A, D, and E).

1.1.1 Traditional methods for oil recovery

Fish oil is a coproduct of fish meal production, which could be produced by two general types of processes: wet-reduction and dry-rendering. The wet-reduction process applies to the processing of fatty species and consists of the following main operations: (1) heating or cooking small whole fish or ground fish offal with live steam under pressure, (2) pressing the cooked fish to release the liquid fraction, the press liquor, and retaining the semi-dry portion, the press cake, (3) screening out the suspended fish solids from the press liquor and returning the wet solids to the press cake, (4) drying the press cake, which was previously fluffed, (5) grinding and sacking the dried meal, (6) heating and centrifuging the screened press liquor to obtain the oil and a aqueous phase, the stickwater, (7) oil purification by centrifuging, (8) recovery of the stickwater, which involves concentration and returning to the press cake.

The dry-rendering process is mainly applied to the production of fish meal from fish or fish offal from nonoily fish. The main steps involved in this process are: (1) coarse grinding of fish offal and (2) treatment of the ground material in a steam-jacketed cooker drier. Sometimes the residual oil may be extracted by pressing.

Thus, the wet-reduction method is particularly suited for the extraction of oil from fatty species and dry rendering is preferred when small quantities of nonoily fish are available [6]. The latter process yields much darker oil than that obtained by the wet-reduction process.

Several other processes for oil extraction involving protein coagulation at temperatures higher than 95 °C were patented [7, 8, 9]. More recently the wet rendering method was used to separate the oil from precooked and non-precooked tuna heads [10]. Higher yields of crude oil were obtained from precooked tuna heads but the oil from non-precooked raw material had higher quality than that from precooked samples. However, crude oil from precooked samples had high DHA (25.5 %) than oil from non-precooked samples (18.8 %).

1.1.2 A modified Conkix process for oil extraction

A new method based on the Conkix process from Alfa Laval for the oil recovery from fish by-products was investigated. This process involves fish mincing, pumping of minced material to a heat exchanger where the fish oil is released and the proteins denaturated, centrifugation in a three-phase decanter, and collection of fish oil. It was used for upgrading

by-products from salmon slaughtering [11]. The salmon oil obtained in this process was not rancid and according to the authors well suited for human consumption or usage. The same process was used for the extraction of herring oil from the by-products resulting from the production of maatjes herring [12].

1.1.3 Oil recovery from the washing mince process

Small fatty fish species represent a significant resource and their utilization for the production of fish minces or surimi has been considered as an alternative to fish meal production. However, the high fat content of these species constitute an important drawback, which led to the development of different approaches to remove fat. One of these processes was tried with capelin [13], which involved cutting the fish into small pieces, washing out the depot fat, dark pigments and viscera under acid (pH 4) or neutral conditions and heating at 40 – 45 °C and bone separation. The maximum release of lipids (*ca.* 80 % of total lipids) at pH 4 was obtained in approximately 60 min. The same amount of lipids was also removed after the same stirring period under neutral conditions when the temperature was adjusted to 40 – 45 °C. This process was also applied in the preparation of snipefish and boar-fish [14].

The process currently used in commercial plants to produce surimi from sardine is similar to the conventional one for lean fish species. However, it presents a difference in the leaching cycle, which involves a first washing step with a cold diluted alkaline solution (0.15 % NaCl, 0.2 % $NaHCO_3$ with an ionic strength of 0.05 and a final pH between 6.8 and 7.3) [15]. The material recovered from this washing step may be leached one or two times in water or 0.2-0.3 % NaCl. Hirata [16] described a method to recover fish oil from highly nutritional fish meat. In this process the sardine mince was ground with diluted alkaline solution (0.125 % $NaHCO_3$, 0.125 % NaCl, water/fish ratio 4:1). The slurry was centrifuged, water added to the floating oil layer resulting from centrifugation and heated up to 40 °C. The mixture was then centrifuged at 10,000xg and collected the oil released. According to the authors, this oil presented a light yellow colour and the amount of volatile compounds was much smaller than that of sardine oil prepared by conventional method at a fish meal plant.

In an alternative processing method to produce surimi from dark-fleshed fatty species [17], the fish muscle is disintegrated into very small particles prior to leaching. The particle reduction is carried out under vacuum to reduce lipid and protein oxidation and also to promote a ready separation of lipids. These authors also used a mixture of sodium pyrophosphate (0.05 – 0.1 %) and bicarbonate (0.1 %) as the leaching medium. The leaching process was done under vacuum, which facilitated the release of water-soluble compounds and lipids. Nishioka *et al.* [18] studied the effect of different vacuum levels, particle size and suction time at 5 mmHg on the fat removal from fish mince. The highest oil removal was achieved in the material with the finest particles (1 mm) at a pressure of 5

mmHg and after 20 min of suction time. The vacuum leaching permitted to remove fat more completely, rapidly and consistently than other methods [19]. Mendes *et al.* [20] reported that the use of vacuum leaching for short time periods was highly efficient and produced a 50 % decrease in fat content of European sardine washed mince when compared with the washing process at atmospheric pressure. The triacylglycerols were extracted the most [21] mainly at intermediate pressure values and increase washing periods. Polyunsaturated fatty acids were the major groups of fatty acids, mainly EPA and DHA, extracted at low pressure levels (4 mmHg) and increased periods of washing. The recovery of sardine by centrifugation of surimi wastewater was described [22]. The wastewater resulting from the surimi preparation was centrifuged continuously by two types of three-phase nozzle centrifuge at 8 – 15 ºC and the oil phase was further dewatered by high-speed continuous centrifuge. The recovered oil showed light yellow colour and a peroxide value less than 1.0 meq/kg. The oil quality indicated that it could be directly used as food materials without further purification.

1.1.4 Oil recovery from acid and alkaline protein solubilization

The application of acid and alkaline digestion methods for the extraction of oil from low oil content fish liver was early described in 1955 [23]. In this type of fish livers the oil is more closely held by the proteins and steaming methods traditionally used for oil extraction from livers with high oil content are not effective. An acid digestion method for oil extraction was reported [6], which is based on a patent by Van Deurs [24]. Later on an alkaline process for fish liver oil recovery was described [25], which intended to yield high vitamin potency oils. In a more recent patented method [26] for the protein recovery involving acid solubilization followed by protein precipitation it is claimed that neutral lipids are also separated during the process. These authors reporting the utilisation of this process to the recovery of proteins from mackerel light muscle referred that 97 % of the initial lipid content were removed [27]. A method for isolation edible protein from animal muscle by solubilizing the proteins in an alkaline aqueous solution was also described [28]. In this process it is claimed that neutral lipids can be separated from the aqueous phase by centrifugation. Kristinsson and Demir [29] using the acid- and alkali-aided processing to prepare protein isolates obtained higher lipid reduction than the conventional surimi processing. The lipid reduction depends on the fish species as well as on the processing alternatives followed in these methods. It was also referred [30] that acid and alkali processes led to a great reduction of membrane and neutral lipids in the protein isolate obtained, which clearly distinguishes from other available processes. In another work [31] was also achieved around 70 % reduction in neutral lipids and about 50 % reduction in membrane phospholipids in the recovery of proteins from herring light muscle by acid or alkaline solubilization processes. Hultin [32] reported that the oil released is richer in saturated fatty acids whereas most of the unsaturated membrane phospholipids are sedimented during centrifugation. Another oil extraction method for sardine

(*Sardinops sagax*) involving pH adjustment was developed [33]. The pH of sardine muscle was adjusted to the isoelectric point (5.5) with either HCl or tartaric or citric acid (with and without calcium) followed by centrifugation. The highest oil recovery was achieved with citric acid plus calcium and the oil extracted showed the best quality and the highest $\omega 3$ PUFA content. The oil extracted by pH adjustment was more stable against oxidation than the oil extracted by the traditional heat extraction method. The beneficial effect of calcium addition in terms of lipid oxidation stability and oil colour was also observed.

1.1.5 Solvent oil extraction

The production of fish protein concentrates (FPC) for human consumption requires the presence of very low oil content (below 1 %, frequently in the vicinity of 0.1 %) in order to make the product palatable for humans. The process of FPC production generally involved an oil extraction step with a solvent. Such as reported [34] this process could be done by employing a standard cycling of a solvent through homogenized fish or using a solvent in an azeotropic distillation-solvent extraction technique. The solvents most successfully used in conventional liquid extraction process are alcohols such as ethanol, propanol or isopropanol [35, 36, 37]. The two-step VioBin process [38] is an azeotropic extraction technique where the first extraction is done with ethylene dichloride and in the second extraction is used isopropanol. In general, organic solvent extraction is effective for lipid removal but it causes protein denaturation and loss of functional properties [39]. On the other hand, the production of FPC requires massive inputs of capital, energy, and expertise [40]. The quality of the recovered oil was not generally mentioned in the many works published on the preparation of FPC because proteins were the main objective. However, it was referred [6] that the oil recovered by solvent extraction was darker than that produced by the wet reduction process and was pointed out [34] that oil recovered in the second extraction in the VioBin process is highly polymerized.

1.1.6 Enzymatic methods for oil recovery

The enzymatic oil extraction from the starting material (whole fish or parts) using food-grade proteases has been indicated as an alternative method because it takes place at moderate temperatures and for a short duration, thus limiting the negative chemical changes occurring in fatty acids during oil recovery by the traditional methods. Proteases are used to disrupt tissues and cell membranes leading to the release of oil.

One of the earliest references to the utilization of enzymatic hydrolysis for oil extraction dates back to the 1930's [41]. The hydrolysis was done with pepsin at pH between 1.2 and 1.5 followed by an alkaline digestion at approximately pH 9. This process was considered

advantageous because it minimized the difficulty of separating the oil from the emulsion formed during the alkaline digestion and reduced the oil acidity due to the inactivation of lipases. However, the need of suitable equipment, of process control and the relatively high costs involved were pointed out as the main disadvantages of the method [23]. Bailey [42] also used proteolytic enzymes under acid conditions to release oil from fish livers with low oil content. The process reduced the oil hydrolysis by lipases and emulsification and allowed to higher oil recovery.

Fish ensiling is a preservation technique first developed in the Nordic countries [43]. In this process the fish liquefaction is due to the activity of endogenous proteases, mainly present in the digestive organs. This autolytic process permitted the oil release, which is removed by centrifugation after heating the autolysate to facilitate oil separation from sludge and dissolved matter [44]. However, it was concluded [45] that the oil recovered from acid medium results in high free fatty acid (FFA) values due to the dissociation of fatty acids salts and breakdown of the oil by a low pH optimum lipase present in some fish species. Moreover, the oil from fish silage is very dark and presents a reduced iodine value [43]. The pigmentation of the oil during ensiling was shown to be caused by the release of haemin, a product resulting from acid hydrolysis of haemoglobin [46]. This author also showed that a 2 % addition of 20 volume hydrogen peroxide inhibit oil pigmentation and reduced FFA content in the recovered oil.

A method to prepare fish muscle tissue with very low lipid content based on the autolysis of skin and belly line was patented [47]. In this process chopped fish is suspended in acidified water which is slowly heated and gently stirred. According to the authors, skin and belly line dissolve completely and the oil released floats to the surface. The enzymic hydrolysis of salmon frames was studied [48] using the commercial protease mixture Protamex™, which has been referred to produce non-bitter hydrolysates. The authors recovered approximately 77 % of total lipids present in the salmon frames, which had 9.3 % EPA and 11.3 % DHA.

The use of enzymatic extraction of fish oil from by-products by Linder *et al.* [49] was patented. These authors [50] also reported the application of three commercial proteases (Alcalase®, Neutrase®, and Flavourzyme™) to extract the oil from salmon heads under mild conditions (below 55 °C). The amount of oil released after two hours of hydrolysis was about 17 % regardless of the enzyme used and was close (20 %) to that obtained by the Bligh and Dyer method [51].

With the objective of obtaining the highest possible amount of valuable products from farmed cod by-products (backbones and liver), it was studied the effect of different hydrolysis conditions on the yield, functional properties and lipid distribution in the various fractions obtained [52]. The enzymatic hydrolysis was performed with Alcalase and it was concluded

that centrifugation forces were important for separation of oily fractions, but not to separate the protein fractions. The results indicated that a two step separation process could be designed, one using low centrifugation speed and a second step at high speed centrifugation to separate oil and emulsion. The major lipid class in the oil and emulsion fractions was triacylglycerols, while phospholipids were found mostly in the protein containing fractions (protein hydrolysate and sludge).

The utilization of various proteases (papain, chymotrypsin, Protamex™, and Flavourzyme®) for the fat extraction of cod by-products (head, backbones, viscera, roes, milt, skin, and cut-offs) was reported in another work [53]. The authors concluded that the use of proteolytic enzymes had generally enhanced the fat extraction, particularly phospholipids.

A number of papers were published in 2005 on the preparation of protein hydrolysates from cod by-products. Thus, it was obtained [54] the highest lipid recovery in the enzymatic hydrolysis (with Flavourzyme or Neutrase) cod by-products when water was not added. The amount of oil fraction obtained with Neutrase was up to 10 % higher compared to Flavourzyme. Similarly, using the same type of raw material and the same enzymes obtained higher oil recovery in the trials without addition of water [55]. They also obtained higher amount of oil released in autolysates with added water than in hydrolysates prepared with commercial enzymes. The highest yields of oil fraction were also obtained in the hydrolysates where Neutrase was used. The presence of digestive tract in the raw material increased the amount of free fatty acids and gave darker colours. The oil fraction contained mostly triacylglycerols and monounsaturated fatty acids. In a last paper [56], was concluded that initial heating of cod by-products causes formation of protein-lipid complexes, which were found in all protein containing fractions being phospholipids and other polar lipids the main part of lipids. The highest amount of separated oil was obtained in the experiments without added water and after initial heating. It was also concluded that the maximum oil and protein hydrolysate yield and the minimum emulsion and sludge yield were achieved in the hydrolysis of unheated raw material with Alcalase and water added.

In another work [57] was reported the use of commercial proteases to extract oil from salmon heads. The oil recovery by the enzymatic method attained 19.6 % whereas the heat treatment led to 14.5 % and the oil released had about 35 % of polyunsaturated fatty acids (PUFA) (7.7 % EPA and 11.9 % DHA). Industrial proteases were also used by Dumay *et al.* [58] to improve lipid and phospholipid recovery from European sardine viscera. The total lipid content in the oily phase and aqueous phases represented 85 % of lipids presented in the raw material indicating that wide-spectrum proteases enhance lipid recovery. It was also concluded that a pre-hydrolysis step led to an increase of total lipids content when compared with the classic organic solvent extraction methods. In another study [59] on the oil recovery from sardine by-products the highest percentage of oil recovery was obtained from the raw

by-products sing Alcalase or Protamex. The latter protease was tested in the hydrolysis of black scabbardfish (*Aphanopus carbo*) [60]. About 36 % of oil was released and 50 - 60 % remained in and emulsion.

1.1.7 Supercritical fluid extraction

The specificity of physicochemical properties of supercritical fluids led to the development of a new process for the extraction of different substances. Supercritical fluids behave as intermediates between liquid and gases, which allow them to dissolving large quantities of natural substances. Such as mentioned [61], the relatively high gas density gives good solvent power, while the relatively low viscosity and high diffusivity provide appreciably higher gas permeability into the solute matrix. Several advantages have been pointed out to this process: (i) gentle treatment of heat-sensitive materials, (ii) solvent-free products covering all legal requirements, (iii) fragrances and aromas remain unchanged, (iv) selective extraction and fractionated separation, and (v) simple solvent recovery. The supercritical fluid extraction (SFE) technique was tested to extract oil from oilseeds and later on for removing lipids from fish muscle proteins. In 1984, Eisenbach [62] reported the effectiveness of the SFE process for fractionating fish oil esters. This technique was also studied for the concentration of n-3 fatty acids [63-69]. The use of this technique for the oil extraction and concentration of n-3-PUFA from fish oil was reviewed [70-73].

The coupled process allowed keeping a high solvent power for SFE-CO2 and also reaching high selectivity levels due to the filtration of supercritical mixtures through a nanofiltration membrane. It was also stated that the coupled separation permitted the concentration of EPA and DHA in the retentate stream and short-chained fatty acids in the permeate.

The supercritical extraction technique has been also used for oil extraction directly from fish muscle although less extensively. This methodology was tested for the removal of lipids from freeze-dried Antarctic krill [74]. As working conditions they used a pressure of 25 MPa at 40 °C, 60 °C and 80 °C. The oil recovered was composed solely by nonpolar lipids, largely triglycerides and presented a relatively high proportion of EPA (11 %). SFE-CO_2 at 4.9 – 24.5 MPa and 40 °C was applied to the lipid extraction of freeze-dried mackerel (*Scomber japonicus*) powder [75]. The yield achieved in the oil extraction increased with pressure and was comparable to that from hot hexane extraction.

The application of SFE-CO_2 for lipid and cholesterol extraction from rainbow trout muscle allow to concluding that: (i) the increase of oil removal with increasing the extraction time from 3 h to 9 h; (ii) the use of ethanol as an entrainer significantly enhanced lipid extraction attaining 97 % but coextraction of polar lipids occurred [61].

This technique was used for the oil removal from freeze-dried Atlantic mackerel (*Scomber scombrus*) muscle [76]. These authors optimized the extraction temperature and pressure. The highest oil yield and concentration of n-3 PUFA was achieved for the temperature range between 35 °C and 55 °C and pressure in the interval 20.7 MPa and 34.5 MPa. According to these authors the high lipid removal and retention of protein functionality in the residue may make this technique useful for future food applications.

This technique was used for the extraction of sardine (*Sardina pilchardus*) oil from freeze-dried muscle [77, 78]. The extraction experiments were done under pressures of 12.6 to 108.0 MPa and temperatures ranging from 40 °C to 48 °C. The loading of sardine oil in CO_2 (kg oil/kg CO_2) increased with increasing pressure, but diminished with increasing temperature. A slight increase in PUFA content of the oil extracted by SFE-CO_2 was observed throughout the extraction.

In a study [79] on the SFE-CO_2 lipid extraction from freeze-dried salmon roe was found that triacylglycerols were not completely extracted and phospholipids PLs not at all. However, the SFE-CO_2 with 10, 15 or 20 % of ethanol as an entrainer was effective in extracting PLs from the above raw material [80]. These authors reported that the extraction with SFE-CO_2 and 20 % ethanol mixture allowed to extracting more than 80 % of the PLs.

The previously reported studies were done in dried fish samples but moisture of the materials may interfere with SFE-CO_2 extraction [81]. Thus, the effect of moisture content on the SFE-CO_2 extractability of oil from Atlantic mackerel was studied [82]. They reported that dehydration of the muscle to 10.2 % (w/w) resulted in high oil yield with minimum changes in protein content and composition.

A mathematical model based on the interaction between oil and water in the fluid phase was developed [83]. This study was done in Atlantic mackerel fillets with different moisture levels prior to extraction at 35 °C and 34.5 MPa. The model developed simulated the extraction behaviour of both oil and water in the initial solubility-controlled region.

The SFE-CO_2 conditions of sardine oil extraction from canning by-products was optimized by Létisse *et al.* [84]. The optimal conditions for pressure, temperature and CO_2 rate were, respectively, 300 bar, 75 °C and 2.5 ml/min during 45 min extraction. The yield of 10.36 % of extracted oil was achieved with an amount of 10.95 % of EPA and 13.01 % of DHA. According to these authors, this technique presented the advantages of a shorter extraction time, prevention of heating and a better organoleptic aspect of the oil by excluding the use of not legally allowed organic solvents.

The extraction of phospholipids from tuna shavings by SFE-CO_2/ethanol was also tried [85]. It was performed at 17.7 MPa and 33 °C and ethanol concentrations higher than 5 % were used. In order to reduce the amount of consumed ethanol in the process, the tuna shavings were previously soaked in the same volume of ethanol before extraction with neat

$SFE\text{-}CO_2$. The extraction was done both at laboratorial and industrial scale and it was concluded that the amount of consumed CO_2 per g tuna shavings decreased with increase in reactor-scale.

This technique was applied to the extraction of squalene from shark liver oil containing around 50 % squalene. Squalene of 95 % purity was obtained without using external reflux [86]. However, the purity could be increased to a maximum of 99 % at fractionation conditions of 250 bar and 60 °C and using reflux of squalene. The extraction and fractionation of a range of crude fish oils (orange roughy oil, deep sea shark and spiny dogfish liver oil and commercial cod liver) using $SFE\text{-}CO_2$ and CO_2+ethanol mixtures was reported [87].

The use of extremely high pressures and high capital costs have been the main disadvantages pointed out [88], which may limit the widespread use of supercritical fluid technique for the oil extraction or PUFA concentration.

2. Oil refining process

The composition and characteristics of crude fish oils depend very much on process and raw material (Table 1). This intermediate product contains a wide range of undesirable compounds, which have to be removed to make the oil meet the quality standards in regard to the obligatory properties for edible or technical applications [89]. Thus, the quality of raw material has to fit the adequate requirements [90] and the process has to be done in a way that essential fish oil components are not being damaged, e.g. polymerisation should be avoided. Within intrinsic materials, small amounts of proteins, water, pigments, free fatty acids (FFAs), phospholipids, and lipid oxidation products are the most significant. Trace components related to environmental pollutants can also be present.

Table 1. Crude fish oil properties and physical characteristics (Adapted from [90]).

Properties	
Moisture and impurities	Usually 0.5 up to 1 %
FAs	Range 1-7 % oleic acid (usually 2-5)
Peroxide value	3-20 meq O_2/kg
Anisidine value	4-60
Totox value	10-60
Iodine value	Capelin (95-160); Herring (115-160); Menhaden (120-200); Sardine (160-200); Anchovy (180-200)
Iron	0.5-7.0 mg/kg
Copper	Less than 0.3 mg/kg
Phosphorus	5-100 mg/kg
Physical characteristics	
Colour (Gardner scale)	Up to 14
Slip melting point	10-15 °C
Boiling point	Greater than 250 °C
Specific gravity	About 0.92 (at 15 °C),
Viscosity	About 60-90 cp (at 20 °C), 20-30 cp (at 50 °C), 10cp (at 90 °C)

The common oil refining is usually made by chemical methods, which include namely settling, and degumming, de-acidification, bleaching, deodorization, and storage and antioxidant addition. In the frame of this purpose hydrogenation is not included.

Settling is made with or without heating, allowing the aqueous phase separation, which is further withdrawn, and in such way part of water, proteinaceous material and phospholipids are purged. Degumming, considered in some circumstances an optional stage, consists of a treatment with water or aqueous solutions of acids or salts (at 30 – 100 ºC) to remove soluble and insoluble impurities that are then separated either by centrifugation or decantation. De-acidification or alkali refining is typically performed with an aqueous alkaline solution to remove FFAs and reduce oil acidity. In addition to the use of alkali substances, supercritical fluid technology, jointly with membrane and enzymatic processes, and thin film deodorisation technologies may be alternatives to the classical procedure [91, 92]. The oil is then heated to 80-85°C and mixed with an activated bleaching clay and/or activated carbon. Sulphurous compounds, soaps, trace metals, and part of the most stable pigments and pigment-breakdown products are separated. Physical adsorption on activated carbon is necessary when the removal of organic contaminants such as dioxins/ furans and dioxin-like PCBs is required [93, 94]. This step also converts hydroperoxides to their respective aldehydes, ketones and other products [95].

The deodorisation step, intended to remove FFAs, aldehydes and ketones, which give unpleasant smell and flavour characteristics to the oil, is conventionally based on the application of high temperatures [96, 97]. The volatile materials are stripped by means of a stripping gas, normally steam at high temperature (190-200 ºC) and low pressure (2-5 mbar). However, the application of such temperatures has to be carefully considered since for temperatures higher than 180 ºC the degradation of n-3 PUFA take place, involving the formation of polymers, isomers, cyclic FAs monomers and other undesirable compounds [98]. Optional methods, such as vacuum steam distillation at low temperatures followed by a treatment in a silica gel column [99], adsorption with a resin [100], or treatment with diatomaceous earth [101] have been considered.

After leaving the deodoriser equipment and be cooled, the fish oil is dosed with the suitable amounts of selected antioxidant(s) and then transferred to appropriate storage tanks; preferably, stainless steel tanks under nitrogen blanketing and at low temperature. To avoid oxidation, improve the stability and extend the fish oil shelf-life, a great number of antioxidants (natural and synthetic compounds) have been proposed [102, 103, 104]. The adequate concentration of antioxidant depends on the chosen substance, the storage conditions and the intended applications. The oxidation of fish oils may also be delayed by encapsulation or microencapsulation with a coating material [105]. For this purpose, various physical and chemical processes using several wall materials have been developed [106],

being spray-drying the most common and cheaper method [107]. Other processes such as freeze drying [108], ultrasonic atomization [109] or coacervation [110] have also been proposed recently as alternative to reduce n-3 PUFA oxidation. Oil-in-water emulsions are increasingly being used as delivery systems for fish oils for functional foods. If properly designed such emulsions can protect the fish oil from lipid oxidation before the addition to foods [111, 112] and in some cases it may also improve the oxidative stability of the fish oil enriched food [113].

2.1 Characteristics of refined fish oils

The chemical and physical characteristics of refined fish, which are influenced by the refining process itself and by the raw material, are quite variable and influence the potential uses and price. Based on information disseminated by [95, 114, 115] and the Codex Standard (1981) some guidelines are given in Table 2.

Table 2. Some quality guidelines for refined fish oils [95, 114, 115].

Parameter	Quality guidelines
Colour	<3.0 Red, 30 Yellow
Odour and taste	Bland
Matter volatile at 105°C	<0.2 %
Insoluble impurities	<0.05 %
Soap content	<0.005 %
Iron	<0.12 mg/kg
Free FAs	<0.10 % (as oleic acid)
Copper	<0.05 mg/kg
Peroxide value	<0.1 meq O_2/kg
Nickel	<0.20 mg/kg

3. n-3 PUFA concentrates

The preparation of n-3 PUFA concentrates usually comprises five steps: de-acidification (optional), transesterification, concentration, deodorisation/earth treatment, and antioxidant addition and fill off. The raw material must have previously undergone a meticulous refining process, including the removal of contaminants [92]. These concentrates may be in different forms: FFAs, methyl and ethyl esters or acylglycerols.

De-acidification is typically the first step, when such operation was not included in the refining strategy. For the most common industrial process, transesterification is the following step, which may be carried out by a chemical catalyst (usually sodium ethoxide at 80-90 °C) or by non-selective lipases [88, 92, 116]. For concentration numerous methods have been anticipated, but only a few are suitable for large-scale production, being the most widespread process the concentration of n-3 PUFA by molecular distillation. Other available methods less frequently used include: adsorption or partitioning chromatography, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction, and urea complexation. Each methodology has its own advantages and drawbacks, and all present important shortcomings that limit their use by the industry. The decision for one specific technology or the combination of two or more must depend on the required final characteristics of n-3 PUFA concentrates and on the costs associated to each choice.

Fractional distillation has been extensively used for partial separation of mixtures of fatty acid esters. Separation is done by asset of their different boiling points. The method applies lower temperatures and shorter heating intervals than conventional distillation methods. Moreover, the use of high vacuum is very common with temperatures ranging from 100-200 °C typically being used during processing in order to minimise heat damage to the oils. Nevertheless, fractionation of marine oil esters has some problems; namely, separation of components becomes less effective with increasing molecular weight, as happens with n-3 PUFA. Moreover, concentration in the natural TAG form (without previous transesterification) presents very important practical difficulties [117].

Adsorption chromatography is based on the potential of some appropriate adsorbents to separate fatty acids (FAs) according to their carbon number or degree of unsaturation. High performance liquid chromatography [118] and silver resin chromatography [119] have been used for the production of n-3 PUFA concentrates. Counter current chromatography and centrifugal partition chromatography have gained much attention in last two decades for isolation of PUFA [120]. In what concerns enzymatic methods, lipases are utilized to catalyze esterification, hydrolysis or exchange of FAs in esters [121]. Accordingly, this technology may enable the performance of the transesterification and concentration phases in a single step. The direction and efficiency of the reaction can be influenced by the choice of experimental conditions and the source of lipase [122]. Low-temperature crystallization is based on the fact that solubility of fats in organic solvents decreases with increasing molecular weight and increases with higher levels of unsaturation [123]. Consequently, at low temperature, long chain saturated FAs which have higher melting points crystallize out and n-3 PUFA remain in the liquid fraction. The low-temperature crystallization process may be carried out in the absence of a solvent or in a selected solvent mixture [88]. The frequently used solvents are methanol and acetone, which pose problems in products for human

consumption. Crystallization in the absence of a solvent occurs by using the Tirtiaux process, which involves slow cooling and slow agitation, and in the hydrophilisation process. SFE-CO_2 is another alternative method for the preparation of n-3 PUFA concentrates [70]. SFE-CO_2 fractionation of fish oil fatty acid ethyl esters was investigated to obtaining enriched n-3 PUFA and a suitable EPA/DHA ratio [72]. The results obtained by these authors indicated that optimizing the extraction conditions could modify the original fatty acid ethyl esters concentrations.

Coupled SFE-CO_2 with nanofiltration separation was used for the concentration of PUFA [73]. Urea complexation by itself or complexation after an enzymatic reaction offers important technical possibilities [49, 124, 125, 126, 127]. This methodology is based on the crystallization of urea in hexagonal structures within the hexagonal crystals, provided the presence of long straight-chain molecules in the medium [128]. The formed channels are sufficiently large to accommodate aliphatic chains. However, whereas straight-chain saturated FAs fit easily in the channels, the presence of double bonds in the carbon chain increases the bulk of the molecule, thereby reducing the likelihood of its complexation with urea [129]. FAs must be previously converted to free FAs (FFAs) or ethyl esters. These forms of the FAs are mixed with an ethanolic solution of urea with some moderate heating. Afterwards, the mixture is allowed to cool down to a particular temperature, depending on the degree of concentration desired [88]. The saturated, monounsaturated and, to a lesser extent, the dienoic FAs are crystallized with urea and the other FAs remain non-crystallized in the solution, being separated by filtration. Among these, a high concentration of n-3 PUFA is found.

Having in mind that concentration may lead to the formation of small amounts of off-odours and leave trace amounts of some impurities, a thorough deodorisation/purification (removal of impurities) process of the oil may be carried out. For this purpose, the concentrated material is heated to 80-85 °C and mixed with an activated bleaching clay and/ or activated carbon. The bleaching clay removes any oxidation products which may be present in the oil along with heavy metals. The addition of activated carbon is required when the removal of organic contaminants such as dioxins/furans and dioxin-like PCB's is still necessary. Another possibility is to promote the contact with powdered or granulated diatomaceous earth [130].

Then, the concentrates have to be stored and stabilized by using similar procedures to those described previously for the refined fish oils.

3.1 Characteristics of n-3 PUFA concentrates

The intensive use of n-3 PUFA concentrates for human health and well-being purposes obliges their characteristics to fulfil a high number of requirements. Thus, all the processing steps must reduce exposure to factors that accelerate oxidation, such as heat, intense light or oxygen. Peroxide values must be kept low throughout all the concentration process. The levels of off-flavours and impurities must be below human sensory perception [92. FFAs must be limited to a minimum because hydrolysis and acidity represent a major loss of quality for n-3 PUFA concentrates. According to the GRAS notifications, concerning food-grade specifications accepted by FDA for different omega-3 concentrates, some quality guideline can be viewed in Table 3 [131, 132].

The form in which concentrates are present is of utmost importance. Effectively, some studies have reported that n-3 PUFA concentrates are better absorbed by human organism and more stable against oxidation when they are in triacylglycerols (TAGs) form than as esters [133, 134]. Moreover, stability is also higher when n-3 PUFA are bound to *sn*-2 position of the glycerol structure than to the *sn*-1,3 position [135]. Therefore, the production of TAGs concentrates with n-3 PUFA in *sn*-2 position and the development of viable technological solutions for this purpose have been acknowledged as a wishful outcome [92].

Table 3. Some quality guidelines for omega-3 FAs concentrates [131, 132].(FDA GRAS Notification 2002; 2006).

Parameter	Quality guidelines
Appearance	Light yellow to yellow at room temperature
Triglycerides	>50 %
Odour and taste	At worst, a slightly fishy odour and taste
Acid value	<1.0 %
Peroxide value	<2.5 meq O_2/kg
p-Anisidine value	<20
Totox value	<25
Density	0.85-1.00 g/ml
Tocopherols	>2.0 mg/g
Moisture	<0.1 %
Unsaponifiables	<2.35 %

4. Structured triacylglycerols

Most of TAGs found in fish oils has a LLL-type structure, which symbolizes three long-chain fatty acids (LCFAs) (16 or more carbon atoms) esterified in the three carbon atoms in the glycerol backbone (named *sn*-1, *sn*-2 and *sn*-3). Once ingested, dietary TAGs are hydrolyzed in the small intestine to *sn*-2 monoacylglycerols (*sn*-2 MAGs) and FFAs released from the *sn*-1,3 positions of the original TAGs due to the action of the human pancreatic lipase, which is *sn*-1,3 specific [136] (Fig. 4).

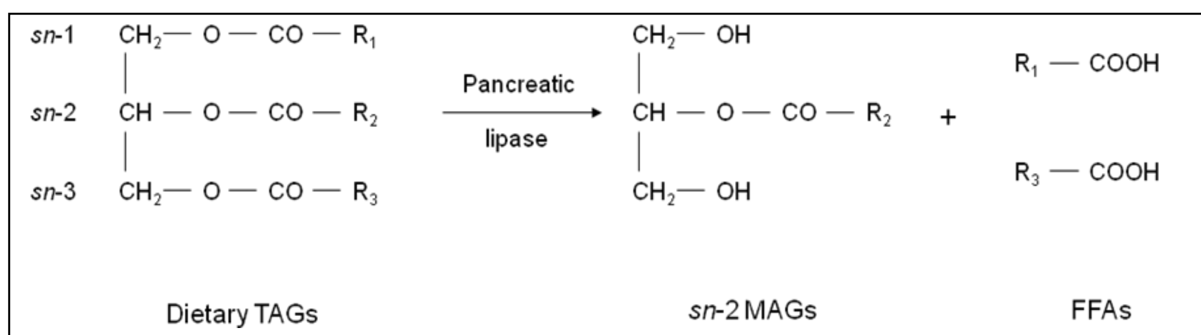


Figure 4. Enzymatic hydrolysis of dietary TAGs by pancreatic lipase. Hydrocarbon chains of FAs esterified in *sn*-1, *sn*-2 and *sn*-3 are represented by R_1, R_2 and R_3 respectively.

Sn-2 MAGs are the most favorable structure for LCFAs to be absorbed by the intestinal mucosa. Thus, allowing these FAs to be used in biosynthetic processes [137]. On the other hand, LCFAs released from *sn*-1 and *sn*-3 positions are mainly transported to the large intestine to form calcium soaps, which are removed with stools. This way, the metabolic use of these FAs is usually impaired.

The hydrolytic activity of pancreatic lipase is higher towards medium-chain FAs (MCFAs) (from 8 to 14 carbon atoms) than for LCFAs, especially PUFA [138]. Therefore, a structured triacylglycerols (STAGs) having a MLM structure (in which “M” refers to a MCFA esterified in *sn*-1,3 positions of the glycerol backbone and “L” refers to a LCFA esterified in *sn*-2 position) offer the most favorable case for the metabolic bioavailability of LCPUFAs, and also is a suitable source of MCFAs, which are transported to the liver to be used as a quick energy source; thus, improved absorption of MLM- type TAGs has been reported [139, 140]. Unfortunately, no natural sources containing MLM-TAGs have been found until now. However, MLM-TAGs can be achieved by means of chemical or enzymatic reactions.

STAGs, generally named structured lipids (SLs), are synthesized molecules, where MCFAs and LCFAs are esterified in the three carbons in the glycerol backbone. MLM-STAGs can be used with alimentary or pharmaceutical purposes. The most employed MCFAs for the STAG synthesis are caprylic acid (8:0) and capric acid (10:0) [141-144]. Most of the experimental designs to obtain STAGs are based in enzymatic reactions, due to its several advantages when compared with chemical synthesis, such as mild reaction conditions, more

biocompatible and safer operative procedures, and the possibility of a more effective control of the FA positional distribution in the final product, due to the use of regiospecific enzymes.

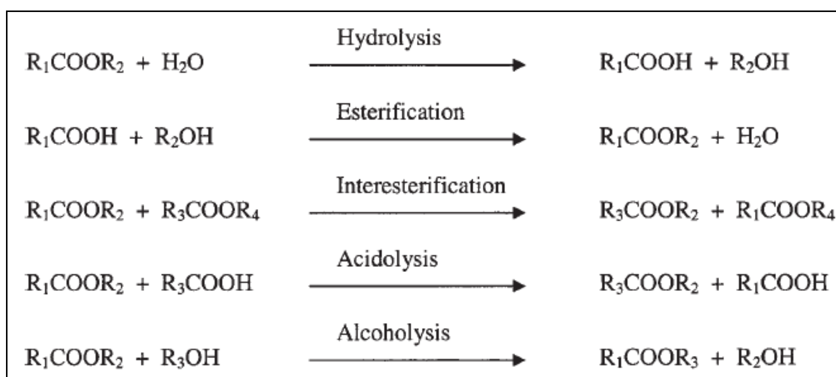
Fish oil constitutes a raw material for STAGs synthesis due to its high content in n-3 PUFA mainly EPA and DHA. The properties of both n-3 PUFAs in the treatment and prevention of a number of pathologies such as coronary heart disease, retinal and brain development, hypertension, rheumatoid arthritis and some types of cancers has been widely reported [145, 146]. Although EPA and DHA are reported to be mainly located in the *sn*-1,3 positions of TAGs of marine mammals, they are usually located in the *sn*-2 position in the TAGs of fish oil [140]. This way, fish oil is a suitable source to be used as a starting material in the synthesis of STAGs containing EPA and DHA in *sn*-2 position. In addition, fish oil has a competitive price compared to other *sn*-2 PUFA rich oils, as single cell oils from algae. Moreover, it seems to be an appropriate way to add value to the by-products generated in the fishery industry.

4.1 Enzymatic Synthesis of STAGs

The synthesis of STAGs containing n-3 PUFA located in the *sn*-2 position, like EPA or DHA, and MCFAs in *sn*-1,3 positions, have received increasing attention in the last years. Thus, as explained above, PUFAs from STAGs can be directly absorbed as *sn*-2 MAGs after lipase pancreatic digestion, resulting in increasing absorption rates and becoming in a target product for alimentary and pharmaceutical industry.

The designing of STAGs with a particular structure requires specific modifications at the desired positions in the glycerol backbone [142]. Although some commercial STAGs have been produced by chemical methods [147], the random nature of the reactions usually limits the possibilities of such procedures. In contrast, the use of *sn*-1,3 regioespecific lipases allows to a positionally specific modification of TAGs. Lipases belong to the hydrolase family, although their physiologic role is the AG hydrolysis, can also operate “in reverse” under low water activity conditions, synthesizing an ester bond. This property has been widely exploited in the production of STAGs, allowing the catalysis of different types of reactions such as interesterification, acydolysis, alcoholisis or esterification (Fig. 5).

Figure 5. Different reactions catalyzed by lipases (adapted from Houde et al. [148]).



Actually, there is a wide supply of regioespecific lipases which show specificity for one or more individual FAs and also present different degrees of regioespecifity. Immobilization supports modify lipase properties, confer thermostability and allow an easy recovery of the biocatalyst, but can also influence a non-desired process of acyl-migration [149], so the choice of a suitable lipase becomes in a great importance step in the design of a STAG synthesis reaction. These processes can be composed of one or more reactions:

4.1.1 One-step processes

Interesterification reaction is indeed an ester exchange where FAs from different TAGs are relocated. Thus, employing a *sn*-1,3 regiospecific lipase, FAs in the extreme positions of the glycerol backbone of an *sn*-2 PUFA rich oil can be replaced by a MCFA, resulting in a mixture of MLM-type STAGs. This kind of reaction has been employed by Xu and coworkers [150], who obtained different products by interesterification of fish oil and a caprylic and capric acid mixture of TAGs, although a percentage of the original PUFAs remained at *sn*-1,3 positions (3.7% of EPA+DHA from total FAs in *sn*-1,3 position).

Acydolisis has been commonly applied looking for a modification of fish oil. In this process, the acyl exchange takes places between an ester and a FA. With this objective, a wide number of works have been reported. Shimada et al. [151] achieved a modified tuna oil with nearly 45% incorporation of caprylic acid mainly located in *sn*-1,3 position (0.5% mol of total FAs in *sn*-2 position) and 14.5% of EPA+DHA in *sn*-2 position, although DHA bounded at *sn*-1,3 position was not exchanged. Similar results were obtained by Zhou et al. [152], who reported a 45.2% incorporation of caprylic acid. Hita el al. [149] indicates an increase in caprylic acid incorporation up to 56% and 26.7% mol EPA+DHA of total FAs in *sn*-2 position when acidolysis of tuna oil was performed, parallel to an increase in the caprylic acid content in *sn*-2 position. Results were improved in a later work [137], when acidolysis of tuna oil in a continuous mode was performed. A 39.3% incorporation of caprylic acid and 42.1% mol of DHA of total *sn*-2 FAs was achieved with an optimal distribution.

4.1.2 Two-step processes

In order to obtain high purity MLM-STAGs from fish oil, two-step enzymatic methods have been developed, involving *sn*-2 MAG production by alcoholysis prior to the final esterification step.

Alcoholysis has been applied by Schmid et al. [153] to different fish oils, performing MAGs with 70% (EPA+DHA), 75% (EPA+DHA) and 50% (EPA) PUFAs. The controversial issue of this method is that purification procedures developed to purify *sn*-2 MAGs used could produce acyl-migration processes.

Muñío et al. [154] reported the production and purification of *sn*-2 MAGs from tuna oil, obtaining a purified fraction of MAGs with 31.8% EPA+DHA, but no regiospecific data were provided. By applying solvent extraction to purify *sn*-2 MAGs, and later an esterification reaction with caprylic acid, acyl-migration seems to be avoided, since PUFA content in *sn*-2 position of STAGs agrees with PUFA content in MAGs, resulting in 39.9% of EPA+DHA of total FAs in *sn*-2 position and 64% incorporation of caprylic acid [155].

Irimescu et al. [156] developed a method for the synthesis of STAG involving ethanolysis of bonito oil and a reesterification of *sn*-2 MAGs with ethyl caprylate. They achieved a final STAG mixture: 85.3% STAGs with two caprylic acid residues, 13% with two original FA residues and 1.7% of tricapryloylglycerol. The calculated 1,3-dicapryloyl-2-docosahexaenoylglycerol to 1,2(2,3)-dicapryloyl-3(1)-docosahexaenoylglycerol ratio was greater than 50:1, showing a good regiospecifity in the whole method.

4.2 STAGs quality and safety

Lipid oxidation is one of the major causes of quality deterioration in lipid-containing foods. Despite their health benefits, all oils containing PUFA are highly susceptible to rapid oxidative deterioration and thus experience problems with storage stability [157]. Therefore, is important to prevent oxidation of any edible oil and foods that contain them in order to maintain their quality and safety.

Oxidation of oils may be initiated by light, heat, and in the presence of metal ions. Oil oxidation occurs via a free-radical chain reaction mechanism involving initiation, propagation, and termination steps [158]. Oxidative deterioration of edible oils involves autoxidation accompanied by both oxidative and nonoxidative reactions [159]. The oxidation products from oils, which include hydroperoxides, decompose to produce a variety of volatile compounds that result in off-flavor and offodor in oils [160].

Peroxidation can be measured by a great number of methods, which include oxygen consumption of PUFAs, lipid hydroperoxide formation, thiobarbituric acid (TBA) trials of malonaldehyde (MDA), gas chromatography/mass spectrometry (GC/MS) of volatile compounds and sensory evolution, and diene conjugation [161].

Rancidity of lipids can be retarded by several procedures. Among them, phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and organic esters of gallic acid (gallates) have been widely used as antioxidants in foods containing oils [159].

Several authors discus about the oxidizability of STAGs. The most interesting results among literature are shown below. The oxidative stability of STAGs based on γ-linolenic acid (18:*3n*-6; GLA)-rich borage oil (BO) and evening primrose oil (EPO), modified with DHA was compared with unmodified borago oil and evening primrose oil as controls [160]. It was probed that STAGs gave rise to higher quantities of conjugated dienes, TBARS, and headspace volatiles as compared to their unmodified counterparts, and results indicated that modified oils were less stable than their unmodified counterparts.

Other work about STAGs pointed in the same direction. This way, after the incorporation of capric acid (CA) into the DHA single cell oil DHASCO, the oxidative stability of the modified DHASCO in comparison with the original DHASCO, as indicated in the conjugated diene values, showed that the unmodified oil remained relatively unchanged during storage for 72 h, but DHASCO-based structured lipid was oxidized to a much higher level than the original oil. It was argued that removal of antioxidants during the process is primarily responsible for the compromised stability of the modified oil [162].

Other studies about STAGs made for infant milk formulation founded that the oxidative stability index, after purification by distillation and addition of antioxidants, was similar between STAGs and the starting oil blends, and also that oxidative stability of the STAGs can be improved with tocopherol addition as antioxidants [163].

After the literature, and taking into account that STAGs may contain FAs with a high degree of unsaturation, to prevent its oxidation it seems advisable to keep the maximum precautions that are usual for PUFAs: the addition of antioxidants, the performing enzymatic synthesis processes in an inert atmosphere, and its preservation at low temperatures.

To conclude STAGs having MLM structure is the best source of s*n*-2 MAGs, which are the most favorable structure for LCFAs, allowing these to be used in biosynthetic processes. Unfortunately, no natural sources containing MLM have been found until now. However, MLM-TAGs can be achieved by means of chemical or enzymatic reactions.

Although some commercial STAGs have been produced by chemical methods, the random nature of the reactions usually limits the possibilities of such procedures. In contrast, the use of *sn*-1,3 regioespecific lipases allows to a positionally specific modification of TAGs. These processes can be composed of one or more reactions. Among one-step processes, acydolisis has been commonly applied looking for a modification of fish oil, while for two-step

processes ethanolysis of bonito oil and a reesterification of the obtained *sn*-2 MAGs with ethyl caprylate showed good results.

Considering that STAGs may contain FAs with a high degree of unsaturation, to prevent its oxidation it seems advisable to keep the maximum precautions that are usual for PUFAs.

5. Fish oil health benefits

Epidemiological studies later confirmed that the marine oil consumed by the Inuit population rich in EPA and DHA, was directly correlated with a cardioprotective role on the risk of cardiovascular disease in healthy individuals [164-168]. Over the years the number of studies in this area has increased, emphasizing the importance of fish oil consumption mainly supported by two important intervention studies that found a cardioprotective effect of these n-3 PUFA, the DART and GISSI studies: (i) DART - Diet and reinfarction trial: a secondary prevention study in which male individuals who had previously suffered a myocardial infarction, ingested over 2 years fish oil capsules, results showed a 29% reduction in overall mortality, and a 33% decrease of deaths caused by heart disease [169]. (ii) The GISSI-Gruppo Italiano per Studio della Sopravvivenza nell it'miocardico infarction –a secondary prevention study with 11,324 patients surviving a myocardial infarction, receiving for 42 months fish oil capsules with 850 mg of EPA + DHA/day. Results pointed out that there was a 15% reduction in death from heart attack or stroke. The overall mortality decreased 21% and sudden death caused by heart disease decreased 45% [170]. Among the possible mechanisms of intake of n-3 PUFA in reducing cardiovascular disease and platelet stability, is described its effect in decreasing the total lipids in plasma, decreased by approximately 25-30% of TAGs in plasma decreased very low density lipoprotein (VLDL) production [165, 171].

Metabolic disorders, like obesity, metabolic syndrome (Met Synd) and diabetes, are characterized by increasing TAG production and impair of TAG metabolism, contributing for VLDL and chylomicron (QM) particle accumulation. Combined, these metabolic features contributed for LDL clearance delay, by LDL-receptor (LDL-R) competition with TAG-rich lipoproteins, lead to long-time LDL circulation. This can take to LDL modified particles towards a small and dense LDL (sdLDL). n-3 PUFA, mainly EPA, enhances β-oxidation, and decreases the substrate available for *neo* TAG synthesis *in vivo*. Both, EPA and DHA, can significantly decrease the VLDL production, by several mechanisms, and n-3-TAG-enriched lipoproteins are more rapidly metabolized by TAG lipases [172-174]. sdLDL, by itself, has less affinity for LDL-R and are more prone to oxidation. Metabolic disorders can also be characterized by an increase in the lipid oxidation, the Maillard reaction and glycoxidation. These effects increase radical-mediated reaction, ROS production, and the accumulation of

very reactive aldehydes and dicarbonyl compounds. This scenario can lead to increasing susceptibility of LDL particles for modifications and oxidation (LDLox), an important biomarker of cardimetabolic risk (Figure 6).

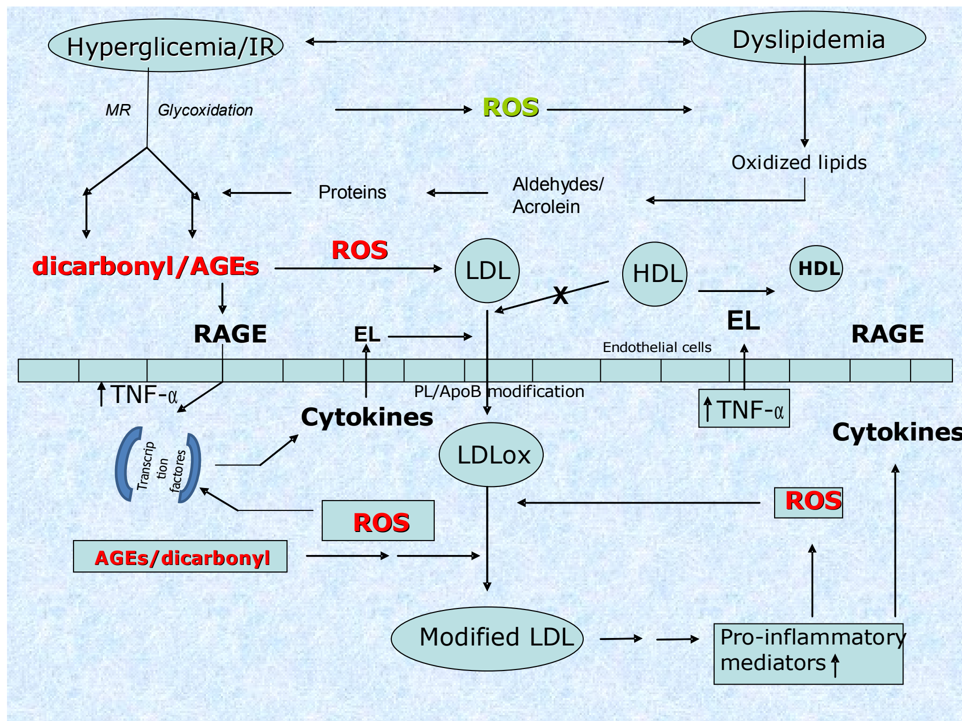


Figure 6. Hyperglicemia and dyslipidemia can lead to increase production of ROS, reactive carbonyl compounds and AGEs, which, by several mechanisms, can impair lipoprotein metabolism and endothelial function. IR: Insulin Resistance; MR: Maillard Reactions; AGEs: Advanced Glycation End Products; RAGE: AGE Receptor; ROS: Reactive Oxygen Species; PL: Phospholipids; EL: Endothelial Lipase.

LDL modified particles also favour the expression of adhesion molecules, increasing macrophages activation and the release of pro-inflammatory mediators, like TNF-α. The later can stimulate transcriptional factors that amplify the production of pro-inflammatory mediators, leading to endothelial cells dysfunction [175-178]. Fish oils ingestion can contribute to a beneficial lipoprotein profile. Moreover, the decrease of TAG-rich lipoproteins effect is well known. However, more accurate evidences of n-3 PUFA mediate lipoprotein effect are needed based on molecular mechanisms. In fact, the majority of studies showed no significant changes in both, total LDL-C and HDL-C. A regular ingestion of fish oil promotes significant changes on HDL2/HDL3 ratio, as well in their phospholipid (PL) composition. The beneficial effects of n-3 PUFA, on lipoprotein profile, can be related with their ability to change the composition, the size or the properties (e.g, antioxidant or cholesterol scavenger capacity) of HDL, and also for LDL particles [179-184]. A shift toward a large HDL and LDL subfractions seems to be beneficial in respect to n-3 PUFA cardioprotective effect, and part of this effect is obtained independently of the changes in the

total HDL-C or LDL-C [179, 181, 184]. A more favourable n-3 PUFA mediated lipoprotein profile is achieved even in those individuals that do not significantly alter the total HDL-C or LDL-C. The role of HDL particles on reverse cholesterol transport is partially dependent of PL composition of these lipoproteins. Likewise, the role of HDL particles in ceramide pathway (inhibiting the sphingosine kinase) seems to be, at least in part, mediated by the type of fatty acids in PL of these lipoproteins [185]. These features support the pivotal role of HDL, and justify the indication for HDL increase/changes (in quantity and/or quality), even in the subjects that achieved a normal LDL-C range [186]. n-3 PUFA can lead to changes in HDL subfractions, increasing large HDL particles and/or reducing small HDL particles. The later seems to be less cardioprotective than large HDL. Some of the effects attributed to n-3 PUFA on lipoproteins can be related to the TAG- and PL-lipase activity. Both, activity and/or expression of LPL (liproteic lipase) and HL (hepatic lipase) can be modulated by n-3 PUFA [187]. However, phospholipase activity might be the key feature in this process. EL (endothelial lipase) is predominantly a phospholipase enzyme, compared with HL and LPL, and has a pronounced effect on HDL metabolism *in vivo*. EL is related to the total HDL-C and with changes of the profile of HDL particles and size [188, 189]. The effect of n-3 PUFA on EL activity and expression can be an important mechanism to understand the effect of n-3 PUFA on HDL (and LDL) subfractions. EPA and DHA can alter the biosynthetic pathways of PL, changing the composition of lipoproteins and EL activity. Moreover, EL showed to possess specificity for different PL and, within the same class, a preference for DHA at *sn*-2 position of PL [190]. The effect of pro-inflammatory mediators on both EL activity and expression seems to be an important mechanism for over-expression of EL that is related with the decrease of the total HDL-C and remodelling of HDL particles molecular composition [189, 191, 192] (Figure 6). EPA and DHA can directly regulate the activity and/or expression of EL; and indirectly, by down-regulating the production of inflammatory mediators [193]. EL has a dual role on lipoprotein metabolism. Despite the effect on HDL remodelling (e.g. PL) and catabolism (Apo A1), the lipase also acts on Apolipoprotein B [189]. HDL Apo A1 catabolism can reduce the protection of LDL particles from oxidation mediated by those apolipoproteins. The effect of n-3 PUFA on ApoAI/ApoB ratio, an important cardiovascular biomarker [194], as well HDL/ApoA1 ratio, can bring some light on the action of these fatty acids related to EL. Future work may explain the potential benefit of n-3 PUFA on lipoprotein metabolism, mediated by direct/indirect effect on EL expression and/or activity.

Studies also pointed out that consumption of n-3 PUFA is related to the relaxation of the endothelium, since the path is vasodilatation dependently enhanced by increased production of NO (nitric oxide), and independently by suppression of the influx of calcium ion through the channel activated by transmembrane potential differences in smooth muscle

cells and endothelium. Inhibition of these channels also contributes to reducing the large and rapid fluctuations in the concentration of free calcium, which contributes to the reduction of ventricular arrhythmias (seen with supplementation with fish oil capsules [195, 196]. Thus, the intake of fish oil (EPA and DHA) is associated with the decrease of blood pressure, this decrease could be related to the inhibition of the renin-angiotensin system, including angiotensin converting enzyme, thereby inhibiting the release of the hormone aldosterone, which is responsible for the increase in blood pressure [197, 198]. Intake of fish oil is also associated with improvements in processes and reduction of atherosclerotic platelet aggregation as well as decreased production of clotting factors and fibrinolytic factors [199-202]. The participation of EPA and DHA in the autonomic nervous system is also evident, since they are associated with decreased activation of the sympathetic system, increasing the activity of the parasympathetic system, thereby reducing cardiovascular risk [203].

Another important effect of fish oil ingestion is related with the impact of n-3 PUFA in membrane fluidity, flexibility, permeability essential for the proper functioning of tissues and organs [204,205]. In fact, n-3 PUFA, particularly EPA and DHA are important structural components of membrane phospholipids, serving as biosynthetic and cellular mediators. DHA is selectively concentrated in the synaptic membrane and retinal membranes and is thought to be related to visual function, brain development, behavior and learning. More recent studies indicate the beneficial effect of n-3 PUFA the neurological level, anti-depressive and mental health [206-208].

n-3 PUFA also act as autacoids, doing regulation of gene expression, the factors of non-nuclear (SREBP- 1c_sterol regulatory element binding protein, isoform 1, subform c) and nuclear transcription (PPARα PPARγ, LXR, and HNF-4α, NF-κ B). The SREBP- 1c is a binding protein, which participates in the activation of the transcription of genes involved in fatty acid synthesis (acetyl-CoA enzyme, acyl-CoA and Δ6, Δ9 and Δ5 desaturases). In the presence of PUFA, the transcription of SREBP- 1c is suppressed, and no interference in the activation process of SRBP- 1c precursor, which occurs in the membrane of the reticulum endoplasmic reducing the activity of this protein in binding to genes encoding the enzymes of fatty acid synthesis [209]. Moreover, the PPARα (peroxisome proliferator activated receptor isoform α), induces the genes for enzymes involved in β-oxidation, and liver desaturases, establishing a direct link to PUFA. When in excess PUFA inhibit the transcription of the desaturase and activate the transcription of the enzymes of β-oxidation of fatty acids [209]. The fatty acid profile of erythrocyte as a biomarker of several pathologic situations has been receiving increasing attention. Dietary intake, digestion, absorption, metabolism, storage and exchange amongst compartments, greatly influence the fatty acid composition in several blood cells, plasma and tissues [210]. Erythrocytes provide a good model for study fatty acid

metabolism [211] and plasma exchanges explains the enrichment of n-3 PUFA in PL-membrane of erythrocytes [212] (Figure 7).

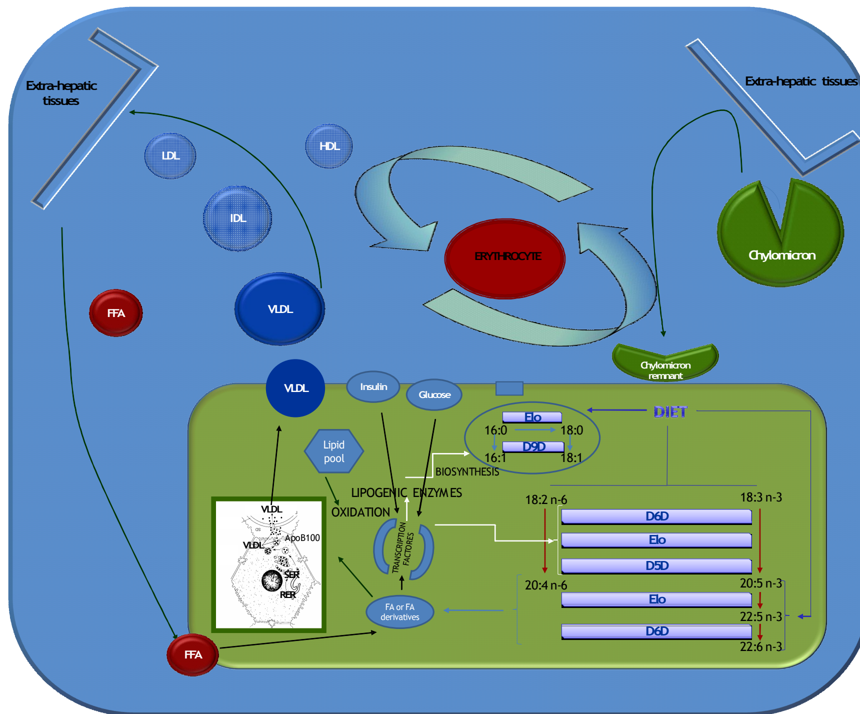


Figure 7. Fatty acids could be incorporated into erythrocytes by exchanges with plasma, as a function of dietary intake, metabolism, and storage and exchange amongst different compartments (adapted for [213]).

Eicosanoid biosynthesis is also a central mechanism in regulating the anti-inflammatory process in body, modulating the intensity and duration of immune response. n-3 PUFA becomes the substrate for the synthesis of prostaglandins, thromboxanes and leukotrienes, responsible for increased vascular permeability, vasodilation and increased production of anti-inflammatory cytokines such as IL-4, IL-10 and TNF-ß and regulation eliminating the production of inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, TNF-α and CRP. Therefore, fish oils seem to have a beneficial effect in diseases such as rheumatoid arthritis, Crohn's disease, cystic fibrosis, obesity, atherosclerosis, among others [197, 214-218].

At normal physiologic conditions a correct balance between inflammatory mediators is the usual situation. The immunomodelation of pro and anti-inflammatory mediators increased in importance in the cases of bowel inflammatory disease, asthma, diabetes, autoimmune diseases or in cancer [219, 220], as well as in obesity and cardiometabolic disorders. The

activation of $CD4^+$ T cells can lead to differentiation of T-helper cells and amplified immune system response. The $CD4^+$ T cells can polarize between the Th1 and Th2 subset as function of different internal and external stimulus. Th1 and Th2 cells release specific cytokines, the first produces mainly IFN-γ, IL-2 and TNF-α [221, 222], that can enhance the pro-inflammatory response by activating the macrophages and increasing the citotoxic capacity of $CD8^+$ T cells. The IFN-γ stimulates B cells to produce antibodies and IL-12, produced by $CD8^+$, DC, and macrophages promote more polarization to Th1 cells by activating STAT4 (signal transducer and activator of transcription 4) [222]. Th2 cells produce mainly IL-4, IL-5, IL-9, IL-10 and IL-13 [219, 222]. IL-4 promotes Th2 cells differentiation, by activation of STAT6, and IL-13, which enhance production of IgG1 and IgE, are related with asthma and allergic reaction. T cells can also differentiate to Th3 cells, that produce TGF-β (transforming growth factor-beta) [222] and have an important role in antigen immunotolerance. The TGF-β can modulate Th1/Th2 balance, macrophages, NK and B cells. This means that TGF-β could have an important role in immunomodelation and inflammation. Dietary components, like n-3 PUFA, can modulate the polarization of T cells by different mechanisms, in both human and animal studies. EPA, DHA and fish oils can 1) reduce the Th1 polarization [219] and provide a better Th1/Th2 balance [223]; 2) decrease IL-1, IL-2, IFN-γ, and TNF-α production [219, 223]; 3) create a more favourable eicosanoids profile; 4) EPA and DHA, or their derivatives, can inhibit transcriptional factor that is involved in pro-inflammatory cascade; and 5) possibly by the increase of TGF-β secretion [224]. This means that n-3 PUFA can produce, locally or systematically a proper environmental by changing Th1/Th2 polarizations, increasing Th3 differentiation, and decreasing pro-inflammatory mediators. The consumption of higher levels of n-3 PUFA, leads to the replacement of ARA in membranes by EPA, including the inflammatory cells. In these cells the incorporation of EPA and DHA is made in a dose-response. This replacement results in decreased inflammatory response due to the formation of eicosanoids 10-100 times less potent than those derived from ARA, increased formation of resolvins E Series with anti-inflammatory action, decreased production of inflammatory cytokines, decreased leukocyte signaling, decreased expression of adhesion molecules like ICAM- 1 and VCAM-1 [214]. Despite the unique properties of EPA and DHA (or derivatives), some of the above mentioned mechanisms could bee integrated in light of n-3/n-6 fatty acids ratio. In addition, immunomodelation of n-3 PUFA can be explained by the capacity to change cellular membrane properties, as a function of their capacity to altering conformation, organization and cellular signalling pathways [219, 223, 225]. Conformational organization of n-3 PUFA acyl chains (Figure 8) alters the properties of the membrane. The change in lipid rafts, constituted mainly by saturated fatty acids, seems to be an important mechanism for anti-

inflammatory and immunomodelation properties of n-3 PUFA [223, 226]. DHA and EPA, modulation of cellular signalling pathways could been explained, at least in partially, by the modification of membrane microdomain organization; reducing NF-κB activation and IL-2 secretion, in $CD4^+$ T cells, by inhibitions of the protein kinase C [227], and of the JNK activity and nuclear factor of activated T cells [228], respectively. The (micro) environmental perpetuation of T cells is an important feature in inflammation and cancer development. Activation of caspase 8, via Fas ligand [226] or TNFRI, induced apoptosis, and is closely related with membrane properties and lipid rafts [229].

a)

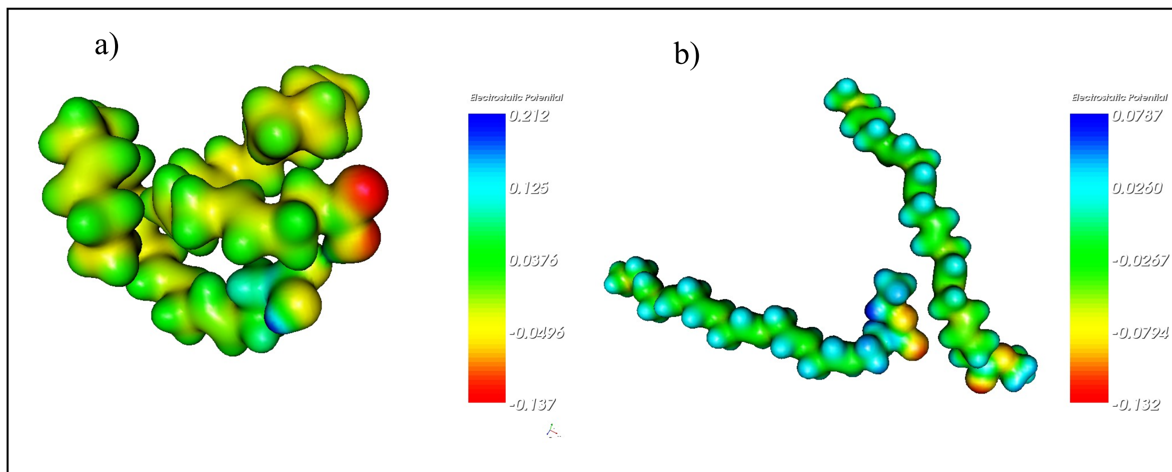


Figure 8. Electrostatic potential for DHA and derivatives. The electrostatic potential (in a.u.) is represented over a constant electronic isodensity ρ (in $Å^{-3}$) surfaces of volume V_s (in $Å^{-3}$). All figures correspond to ρ = 0.05 $Å^{-3}$. (a) DHA dimer, (b) DHA ester dimer.

6. Recommended Intake Levels of n-3 PUFA and n-3 Sources in the Diet

Current recommendations for the intake of n-3 PUFA (18:3n-3 including, EPA and DHA) were established for the prevention of deficiency in EFA, and the prevention and treatment of CVD. Thus, the WHO recommended for the prevention of CHD and stroke ischaemia, the PUFA in the diet should represent 60-10% of total energy intake, being 5-8% n-6 PUFA and 1-2% n-3 PUFA, as well as a regular consumption of 1-2 servings of oily fish per week, representing 400-1000 mg of EPA + DHA / day [230]. The ISSFAL (International Society for the Study of Fatty Acids and Lipids) recommended that the maintenance of cardiovascular health that LA represents 2% of energy, LNA 0.7% of the energy and 0.5 g of EPA + DHA / day [231]. The AHA (American Heart Association) recommended in 2006 that the American people should eat two portions (85 g) of oily fish a week, about 400-500 mg of EPA + DHA/day, and to secondary prevention should use 1g of EPA + DHA /day [232, 233].

However the intake of more than 3 g of n-3 PUFA / day appear to be responsible for gastrointestinal disorders [234]. Supplementation with very high n-3 PUFA levels (> 7g/dia)

may generate excessive bleeding when the level of n-6 PUFA in the diet is low (ARA, EPA 4:1), suppress certain immune responses, increase cholesterol (especially in individuals with familial combined hyperlipidemia) or cause damage oxidative they contain low levels of vitamin E [235, 236], and can transiently increased glycemia, by enhancing neoglucogenic subtracts [237] and/or increased β-oxidation.

References

1. Barlow, S. (2003). World market overview of fish meal and fish oil. In Peter J. Bechtel (Ed.), Advances in Seafood Byproducts: 2002 Conference Proceedings (pp. 11-25). Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks.
2. Chamberlain, A. (2011). Fishmeal and fish oil – The facts, figures, trends, and IFFO's responsible supply standard. International Fishmeal & Fish Oil Organisation. http://www.iffo.net/downloads/Datasheets%20Publications%20SP/FMFOF2011.pdf (5th April 2011)
3. Bergé, J.-P. & Barnathan, G. (2005). Fatty acids from lipids of marine organisms: Molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In Y. Le Gal and R. Ulber (Eds.), Marine Biotechnology I (pp. 49-125). Springer-Verlag Berlin.
4. Shahidi, F. (2007). Maximizing the value of marine by-products: an overview. In F. Shahidi (Ed.), Maximising the value of marine by-products (pp. xxi-xxv). Woodhead Publishing Limited, Cambridge, England.
5. Hjaltason, B. (1990). New products, processing possibilities and market for fish oil. In Proceedings of the International By-products Conference, Anchorage, Alaska, USA, 131.
6. Brody, J. (1965). Fishery By-Products Technology. The Avi Publishing Company, Inc. 232 p.
7. Takahashi, C. & Mitsui, K. (1923). Improvements relating to the extraction of oils from fish. British Patent GB199054.
8. Fawcett, E. W. (1935). Process of refining crude fats and fatty oils. British Patent GB438056.
9. Fosse, J. (1947). Improved apparatus for the heat treatment of animal material, especially from whale, herring or other fishes for the production of oil and dry stuff. British Patent GB590638.
10. Chantachum, S., Benjakul, S. & Sriwirat, N. (2000). Separation and quality of fish oil from precooked and non-precooked tuna heads. Food Chemistry, 69: 289-294.
11. Skåra, T. & Cripps, S. (1997). Upgrading of waste from salmon slaughteries using a three phase separation process. In Luten, J. B., Børrensen, T., Oehlenschläger, J. (Eds.), Seafood From Producer to Consumer, Integrated Approach to Quality. Elsevier Science, B. V.: Amsterdam, The Netherlands, p. 103-111.

12. Aidos, I. (2002). Production of high-quality fish oil from herring byproducts. Ph. D. Thesis, Wageningen University, The Netherlands, 205 p.
13. Eide, O., Børresen, T. & Strøm, T. (1982). Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from small fatty fish species. J. Food Sci., 47, 347-349, 354.
14. Nunes, M. L. (1983). Minces from snipefish *Macroramphosus* spp and boar-fish *Capros aper* gutted and cleaned by a chemical and physical method. XVII International Congress of Refrigeration, Commission C2. Paris
15. Shimizu, Y., Toyohara, H. & Lanier, T. C. (1992). Surimi Production from Fatty and Dark-Fleshed Fish Species. In T. C. Lanier and C. M. Lee (eds), Surimi Technology (pp. 181-207), Marcel Dekker, Inc., New York.
16. Hirata, F., Saeki, H., Nonaka, M., Kawasaki, K., Ooizumi T. & Motoe, K. (1993). Recovery of fish oil from the manufacturing process of highly nutritional fish meat for foodstuffs from sardine. Nippon Suisan Gakkaishi, 59, 111-116.
17. Tokunaga, T. & Nishioka, F. (1988). Surimi production from fatty Japanese sardines. In Proc. Intl. Conf. Fatty Fish Utilization: Upgrading from Feed to Food (p. 143). Raleigh NC, UNC Sea Grant Publ. 88-04.
18. Nishioka, F., Tokunaga, T., Fujiwara, T. & Yoshioka, S. (1990). Development of new leaching technology and a system to manufacture high quality frozen surimi. In Chilling and freezing of new products (pp. 73-80). International Institute of Refrigeration Commission C2, Aberdeen.
19. Lanier, T. C. (1994). Functional fod protein ingredients from fish. In Z. E. Sikorski, B. S. Pan, and F. Shahidi (Eds) Seafood proteins (pp. 127-159). Chapman and Hall, New York.
20. Mendes, R., Gómez-Guillén, C. & Montero, P. (1997). Effect of a new vacuum leaching technology on the textural characteristics of sardine mince. Z Lebens Unters Forsch A, 204, 113-120.
21. Mendes, R., Vital, R., Bandarra, N. M., 2000. Effect of a vacuum-leaching technology on the proteins and lipids of lean sardine (*Sardina pilchardus*) mince. Eur Food Res Techol, 212, 31-38.
22. Toyoshima, K., Noguchi, R., Hosokawa, M., Fukunaga, K., Nishiyama, T., Takahashi, R. & Miyashita, K. (2004). Separation of sardine oil without heating from surimi waste and its effect on lipid metabolism in rats. J. Agric. Food Chem., 52, 2372–2375.
23. Butler, C. (1955). The fish liver oil industry. U. S. Fish and Wildlife Service. Fishery Leaflet 233.
24. Van Deurs, J. A. S. (1931). Process for treating fatty raw materials. U. S. Pat. No. 1,833,061.

25. Aure, L. (1967). Manufacture of fish-liver oil. In M. E. Stansby (Ed.) Fish oils. Their chemistry, Technology, Stability, Nutritional Properties, and Uses (pp. 193-205). The AVI Publishing Company, Inc.
26. Hultin, H. O. & Kelleher, S. D. (1999). Process for isolating a protein composition from a muscle source and protein composition. US Patent 6,005,073.
27. Hultin, H. O. & Kelleher, S. D. (2000). High efficiency alkaline protein extraction. U. S. Patent 6,136,959.
28. Hultin, H. O.& Kelleher, S. D. (2000). Surimi processing from dark muscle fish. In Jae W. Park (Ed.), Surimi and Surimi Seafood (pp. 59-77). Marcel Dekker, Inc., New York.
29. Kristinsson, H. G. & Demir, N. (2003). Functional protein isolates from underutilized tropical and subtropical fish species and byproducts. In P. Bechtel (Ed.), Advances in Seafood Byproducts (pp. 277-298). Alaska Sea Grant College Program, University of Alaska, Anchorage.
30. Kristinsson H. G., Theodore, A. E. & Ingadottir, B. (2007). Chemical processing methods for protein recovery from marine by-products and underutilized species. In F. Shahidi (Ed.), Maximising the value of marine by-products (pp. 144–168). Woodhead Publishing Limited, Cambridge, England.
31. Undeland, I., Kelleher, S. D. & Hultin, H. O. (2002). Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J. Agric. Food Chem., 50, 7371-7379.
32. Hultin, H. O. (2002). Recent Advances in Surimi Technology. In M. Fingerman and R. Nagabhushanan (Eds.) Recent Advances in Marine Biotechnology (pp. 241-251). Volume 7. Seafood Safety and Human Health, Science Publishers, Inc., Enfield (NH), USA.
33. Okada, T. & Morrissey, M. T. (2007). Recovery and characterization of sardine oil extracted by pH adjustment. J. Agric. Food Chem., 55, 1808–1813.
34. Pigott, G. M. (1967). Production of fish oil. In M. E. Stansby (Ed.), Fish oils. Their chemistry, Tecnohology, stability, nutritional properties, and uses (pp. 183–192). The Avi Publishing Company, Inc.
35. Anon. (1966a). Extraction process wins protein from fish. Chem. Eng., 73, 98–99.
36. Anon. (1966b). Marine protein concentrate. Fishery Leaflet 584. U.S. Fish & Wildlife Service, Washington, D. C.
37. Windsor, M. L. (1977). Fish protein concentrate. Torry Advisory Note No. 39. Torry Research Station, 10 p.
38. Levin, E. & Finn, R. K. (1955). A process for dehydrating and defatting tissues at low temperature. Chem. Eng. Progr., 51, 223–226.

39. Pariser, E. R., Wallerstein, M. B., Corkery, C. J. & Brown, N. L. (1978). In Fish protein concentrate Panacea for Protein Malnutrition? MIT Press, Cambridge, Mass. 296 pp.
40. Pariser, E. R. & Wallerstein, M. B. (1980). Fish protein concentrate - lessons for future food supplementation. Food Policy, 5, 298–305.
41. Brocklesby, H. N. & Green, K. (1937). Variations in vitamin A content of liver oils in the grayfish. Fisheries Res. Board Can., Ottawa.
42. Bailey, B. E. (1952). Digestion methods in the production of marine oils. Marine Oils Fish. Res. Bd Canada, Bulletin No. 89, pp. 195–197.
43. Tatterson, I. N. & Windsor, M. L. (1974). Fish silage. J. Sci. Fd Agric., 25, 369–379.
44. Raa, J., Gildberg, A. & Strøm, T. (1983). Silage production – theory and practice. In D. A. Ledward, A. J. Taylor & R. A. Lawrie (Eds), Upgrading waste for Feeds and Food (pp. 117–132). Butterworths, London.
45. Reece, P. (1980). Control and reduction of free fatty acid concentration in oil recovered from fish silage prepared from sprat. J. Sci. Food Agric., 31, 147–155.
46. Reece, P. (1981). Recovery of high quality oil from mackerel and sprat by the silage process. J. Sci. Food Agric., 32, 531–538.
47. Eide, O., Raa, J., Strøm, T. & Tiedemann, E. (1979). Norwegian Patent 791481.
48. Liaset, B., Julshamn, K. & Espe, M. (2003). Chemical composition and theoretical evaluation of the produced fractions from enzymic hydrolysis of salmon frames with Protamex™. Process Biochemistry, 38, 1747-1759.
49. Linder, M., Fanni, J. & Parmentier, M. (2002). Procédé d’extraction d’huile par voie enzymatique et obtention d’hydrolysats protéiques à fonctionnalités dirigées. Brevet FR 2.835.703.
50. Linder, M., Fanni, J. & Parmentier, M. (2005). Proteolytic extraction of salmon oil and PUFA concentration by lipases. Marine Biotechnology, 15, 70-76.
51. Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.
52. Šližytė, R., Nguyen, J. V., Rustad, T. & Storrø, I. (2004). Hydrolysis of cod (*Gadus morhua*) by-products: Influence of initial heat inactivation, concentration and separation conditions. J. Aquatic Food Prod. Technol., 13, 31-48.
53. Dumay, J., Barthomeuf, C. & Bergé, J. P. (2004). How enzymes may be helpful for upgrading fish by-products: Enhancement of fat extraction. J. Aquatic Food Prod. Technol., 13: 69-84.
54. Šližytė, R., Daukšas, E., Falch, E., Storrø, I. & Rustad, T. (2005). Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochemistry, 40, 1415–1424.

55. Daukšas, E., Falch, E., Šližytė, R. & Rustad, T. (2005). Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochemistry, 40, 2659-2670.
56. Šližytė, R., Rustad, T. & Storrø, I. (2005). Enzymatic hydrolysis of cod (*Gadus morhua*) by-products. Optimization of yield and properties of lipid and protein fractions. Process Biochemistry, 40, 3680 – 3692.
57. Gbogouri, G. A., Linder, M., Fanni, J. & Parmentier, M. (2006). Analysis of lipids extracted from salmon (*Salmo salar*) heads by commercial proteolytic enzymes. Eur. J. Lipid Sci. Technol., 766-775.
58. Dumay, J., Donnay-Moreno, C., Barnathan, G., Jaouen, P. & Bergé, J.P. (2006). Improvement of lipid and phospholipid recoveries from sardine (*Sardina pilchardus*) viscera using industrial proteases. Process Biochemistry, 41, 2327–2332.
59. Batista, I., Ramos, C., Mendonça, R. & Nunes, M. L. (2009). Enzymatic hydrolysis of sardine (*Sardina pilchardus*) by-products and lipid recovery. Journal of Aquatic Food Product Technology 18, 120-134.
60. Batista, I., Ramos, C., Coutinho, J., Bandarra, N. M. & Nunes, M. L. (2010). Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products and antioxidative activity of the hydrolysates produced. Process Biochemistry, 45: 18–24.
61. Hardardottir, I. & Kinsella, J. E. (1988). Extraction of lipid and cholesterol from fish muscle with supercritical fluids. J. Food. Sci., 53, 1656-1658, 1661.
62. Eisenbach, W. (1984). Supercritical fluid extraction: a film demonstration. Ber. Bunsenges. Phys. Chem., 88: 882-887.
63. Krukonis, V. J. (1988). Processing with supercritical fluids: overview and applications. In B. A. Charpentier and M. R. Sevenants (Eds.), Supercritical Fluid Extraction and Chromatography: Techniques and Applications (pp. 26–43), B. A., ACS Symp. Series No. 366, American Chemical Society, Washington, DC.
64. Rizvi, S. S. H., Chao, R. R. & Liew, Y. J. (1988). Concentration of omega-3 fatty acids from fish oil using supercritical carbon dioxide. In B. A. Charpentier and M. R. Sevenants (Eds.), Supercritical Fluid Extraction and Chromatography: Techniques and Applications (pp. 89–108). ACS Symp. Series No. 366, American Chemical Society, Washington, DC.
65. Nilsson, W. B., Gauglitz, E. J. & Hudson, J. K. (1989). Supercritical fluid fractionation of oil esters using incremental pressure programming and temperature gradient supercritical fluid CO_2. J. Am. Oil Chem. Soc., 66, 1596-1600.
66. Nilsson, W. B., Gauglitz, E. J., Hudson, J. K., Stout, V. F. & Spinelli, J. (1988). Fractionation of menhaden oil ethyl esters using supercritical fluid CO_2. J. Am. Oil Chem. Soc., 65, 109-117.

67. Nilsson, W. B., Seaborn, G. T. & Hudson, J. K. (1992). Partition coefficients for fatty acid esters in supercritical CO_2 with and without ethanol. J. Am. Oil Chem. Soc., 69, 305-308.
68. Higashidate, S., Yamauchi, Y. & Saito, M. (1990). Enrichment of eicosapentaenoic acid and docosahexaenoic acid esters fro esterified fish oil by programmed extraction-elution with supercritical carbon dioxide. J. Chromatogr., 515: 295–303.
69. Staby, A. & Mollerup, J. (1993). Solubility of fish oil fatty acid ethyl esters in sub- and supercritical carbon dioxide. J. Am. Oil Chem. Soc., 70, 583-588.
70. Mishra, V. K., Temelli, F. & Ooraikul, B., 1993. Extraction and purification of ω3-fatty acids with an emphasis on supercritical fluid extraction, a review. Food Res. Intern., 26, 217-226.
71. Riha, V., G. Brunner, G., 2000. Separation of fish oil ethyl esters with supercritical carbon dioxide. J. of Supercritical Fluids, 17: 55-64.
72. Perretti, G., Motori, A., Bravi, E., Favatic, F., Montanari, L. & Fantozzi, P. (2007). Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. J. of Supercritical Fluids, 40: 349-353.
73. Sarrade, S. J., Rios, G. M. & Carlès, M. (1998). Supercritical CO_2 extraction coupled with nanofiltration separation. Applications to natural products. Separation and Purification Technology, 14: 19 – 25.
74. Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M. & Hata, K. (1986). Supercritical carbon dioxide extraction of oils from Antarctic krill. J. Agric. Food Chem., 34, 904-907.
75. Ikushima, Y., Saito, N., Hatakeda, K., Ito, S., Asano, T. & Goto, T. (1986). A supercritical carbon dioxide extraction from mackerel (*Scomber japonicus*) powder: Experiment and modelling. Bull. Chem. Soc. Jpn., 59: 3709–3713.
76. Temelli, F., LeBlanc, E. & Fu, L. (1995). Supercritical CO_2 extraction of oil from Atlantic mackerel (*Scomber scombrus*) and protein functionality. J. Food. Sci., 60, 703-706.
77. Bandarra, N. M., Fontan, I., Esquível, M. M., Bernardo-Gil, M. G., Batista, I., Nunes, M. L. & Empis, J. M. A. (1995). Characterisation of sardine oil extracted by supercritical carbon dioxide and classical methods. Proceedings of the Conference "Current Status and Future Trends in Analytical Food Chemistry" EURO FOOD VII, Vienna, 18-20 September 1995. p. 219-223.
78. Esquível, M. M., Bandarra, N. M., Fontan, I., Bernardo-Gil, M. G., Batista, I., Nunes, M. L. & Empis, J. A. (1997). Supercritical carbon dioxide extraction of sardine *Sardina pilchardus* oil. Lebensm.-Wiss. u.-Technol., 30, 715-720.
79. Tanaka, Y. & Ohkubo, T. (2003). Extraction of lipids from salmon roe with supercritical carbon dioxide. Journal of Oleo Science, 52, 295-301.

80. Tanaka, Y., Sakaki, I. & Ohkubo, T. (2004). Extraction of phospholipids from salmon roe with supercritical carbon dioxide and an entrainer. Journal of Oleo Science, 53, 417-424.
81. King, J.W., Johnson, H.J., Friedrich, J.P., 1989. Extraction of fat tissue from meat products with supercritical carbon dioxide. Journal of Agricultural Food Chemistry 37, 951–954.
82. Dunford, N. T., Temmeli, F. & LeBlanc, E. (1997). Supercritical CO_2 extraction of oil and residual proteins from Atlantic mackerel (*Scomber scombrus*) as affected by moisture content. J. Food Sci., 62, 289-294.
83. Dunford, N.T.; Goto, M.; Temmeli, F. (1998). Modelling of oil extraction with supercritical CO_2 from Atlantic mackerel (*Scomber scombrus*) at different moisture content. J. of Supercritical Fluids, 13: 303-309.
84. Létisse, M., Rozières, M., Hiol, A., Sergent, M. & Comeau, L. (2006). Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier. I. Optimization of extraction conditions. J. of Supercritical Fluids, 38: 27-36.
85. Tanaka, Y. & Sakaki, I. (2005). Extraction of phospholipids from unused natural resources with supercritical carbon dioxide and an entrainer. Journal of Oleo Science, 54, 569-576.
86. Catchpole, O. J., von Kamp, J.-C. & Grey, J. B. (1997). Extraction of squalene from shark liver oil in a packed column using supercritical carbon dioxide. Ind. Eng. Chem. Res., 36: 4318-4324.
87. Catchpole, O. J., Grey, J. B. & Noermark, K. A. (2000). Fractionation of fish oils using supercritical CO_2 and CO_2+ethanol mixtures. J. of Supercritical Fluids, 19: 25-37.
88. Shaihidi, F. & Wanasundara U. N. (1998). Omega-3 fatty acid concentrates: nutritional aspects and production technologies. Trends in Food Science & Technology, 9, 230-240.
89. FAO (1986). The production of fish meal and oil. FAO Fisheries Technical Paper, vol. 142. FAO, Rome (Italy), 61 p.
90. EFSA (BIOHAZ - Working Group on fish oil for human consumption: Sava Buncic, Charlotte Jacobsen, Maria Leonor Nunes, Jörg Oehlenschläger and Miguel Prieto Maradona) 2010. Scientific Opinion on Fish Oil for Human Consumption. Food Hygiene, including Rancidity. Panel on Biological Hazards. EFSA Journal, 8, 1874-1922.
91. Bhosle, B. M. & Subramanian, R. (2005). New approaches in deacification of edible oils-A review. J. Food Eng, 69, 481−494.
92. Rubio-Rodríguez, N., Beltrán, S., Jaime, I., de Diego, S. M., Sanz, M. T. & Carballido, J. R. (2010). Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innov Food Sci Emerg Technol, 11, 1-12.

93. Maes, J., de-Meulenaer. B., van-Heerswhyngels, P., de-Greyt, W., de-Pauw, E. & Huyghebaert, A. (2005). Removal of dioxins and PCB from fish oil by activated carbon and its influence on the nutritional quality of the oil. J Am Oil Chem Soc, 82, 593-597.
94. Oterhals, A., Solvang, M., Nortvedt, R. & Berntssen, M. H.G. (2007). Optimization of activated carbon-based decontamination of fish oil by response surface methodology. European J Lipid Sci Technol, 109, 691-705.
95. Hamm. W. (2009). Processing of fish oil. In B. Rossell (Ed.), Fish oils, pp. pp. 81-98). UK: Wiley-Blackwell.
96. Čmolik, J. & Pokorný, J. (2000). Physical refining of edible oils. European J Lipid Sci Technol, 102, 472-486.
97. Aidos, I., van-der-Padt, A., Boom, R. M., & Luten, J. B. (2003). Quality of crude fish oil extracted from herring by-products of varying states of freshness. J Food Sci, 68, 458-465.
98. Fournier, V., Destaillats, F., Juanéda, P., Dionisi, F., Lambelet, P., Sébédio, J. L. & Berdeaux, O. (2006). Thermal degradation of long-chain polyunsaturated FAs during deodorization of fish oil. European J Lipid Sci Technol, 108, 33-42.
99. Chang, S. S., Bao, Y. & Pelura, T. J. (1989). Purification of fish oil. Patent U.S. 4.874.629.
100. Nishimoto, Y. & Nagagawa, M. (1996). Method for refining fish oil. Patent JP 08311481.
101. Yoshikazu, T., Hirosi, Y., Kazuko, H. & Satoru, K. (1997). Method for refining and manufacturing fats and oils containing polyunsaturated FAs. European Patent 0773283.
102. Kamal-Eldin, A. & Yanishlieva, N. V. (2002). N-3 FAs for human nutrition: stability considerations. European J Lipid Sci Technol, 104, 825-836.
103. Jacobsen, C., Let, M.B., Nielsen, N.S. & Meyer, A.S. (2008). Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: A comparative evaluation. Trends Food Sci Technol, 19/2: 76-93.
104. Jiménez-Alvárez, D., Giuffrida, F., Golay, P. A., Cotting, C., Lardeau, A. & Keely, B. J. (2008). Antioxidant activity of oregano, parsley, and olive mill wastewaters in bulk oils and oil-in-water emulsions enriched in fish oil. J Agri Food Chem, 56, 7151-7159.
105. Matsuno, R. & Adachi, S. (1993). Lipid encapsulation technology – Techniques and applications to food. Trends Food Sci Technol, 4, 256-261.
106. Desai, K. & Park, H. J. (2005). Recent developments in microencapsulation of food ingredients. Drying Technol, 23, 1361-1394.
107. Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res Internat, 40, 1107-1121.

108. Heinzelmann, K., Franke, K., Velasco, J., & Márquez-Ruíz, G. (2000). Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. European Food Research and Technol, 211, 234-239.

109. Klaypradit, W. & Huang, Y. W. (2008). Fish oil encapsulation with chitosan using ultrasonic atomizer. Lebensmittel-Wissenschaft Technologie, 41, 1133-1139.

110. Barrow, C. J., Nolan, C. & Holub, B. J. (2009). Bioequivalence of encapsulated and microencapsulated fishoil supplementation. J Functional Foods, 1:38-43.

111. Day, L., Xu, M., Hoobin, P., Burgar, I. & Augustin, M. A. (2007). Characterisation of fish oil emulsions stabilised by sodium caseinate, Food Chem, 105, 469–479.

112. McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components, J. Food Sci, 72, 109–124.

113. Let, M. B., Jacobsen, C. & Meyer, A. S. (2007). Lipid oxidation in milk, yoghurt and salad dressing enriched with neat fish oil or pre-emulsified fish oil. J Agric Food Chem, 55, 7802-7809.

114. Bimbo, A. P. (1998). Guidelines for characterizing food-grade fish oils. Inform, 9, 473-483.

115. Codex Standard nº 19, (1981). Codex Standard for edible fats and oils not covered by individual standards. P. 1-5 (http://www.codexalimentarius.net/web/standard_list.jsp, retrieved in May 2011.

116. Akoh, C. C. (1993). Lipase-catalysed synthesis of partial glyceride. Biotechnol Lett, 15, 949.

117. Stout, V. F., Niisson, W. B., Krzynowek, J. & Schlenk, H. (1990). Fractionation of Fish Oil and Their FAs. In M. E. Stansby (Ed.), Fish Oils In Nutrition (pp. 73-119). New York (USA): Van Nostrand Reinhold.

118. Beebe, L. M., Brown, P. R. & Turcotte, L. G. (1988). Preparative-scale-high-performance liquid chromatography of omega-3 polyunsaturated fatty acid esters derived from fish oil. J Chromatography, 495, 369-378.

119. Adlof, R. O. & Emiken, E. A. (1985). The Isolation of omega-3 polyunsaturated FAs and methyl esters of fish oils by silver resin chromatography. J Am Oil Chem Soc, 62, 1592-1595.

120. Goffic, F.L., (1997). Countercurrent and centrifugal partition chromatography as new tools for preparative-scale lipid purification. Lipid Technol, 9:148-150.

121. Marangoni, A. & Rousseau, D., (1995). Engineering Triacylglycerol: the Role of Interesterification. Trends Food Sci Technol, 6, 329-335.

122. Yadwad, V. B., Ward, O. P. & Noronha, L. C. (1991). Application of lipase to concentrate the docosahexaenoic acid fraction of fish oil. Biotech Bioeng, 38, 956-959.

123. Chawla, P. & deMan, J. M. 1990. Measurement of the size distribution of fat crystals using a laser particle counter. J Am Oil Chem Soc, 76, 329-332.
124. Kojima, Y., Sakuradani, E. & Shimizu, S. (2006). Different specificity of two types of *Pseudomonas lipases* to C20 FAs with Δ5 unsaturated double bond and their application for selective concentration of FAs. J Biosci Bioeng, 101, 496–500.
125. LeGoffic, F., Nguyen, A., & Khayat-Frydman, C. (2000). Concentration and purification of polyunsaturated fatty acid esters by distillation enzymatic transesterification coupling. WO 00/73254.
126. Lin, T.J., Chen, S. W. & Chang, A. C. (2006). Enrichment of n−3 PUFA contents on triglycerides of fish oil by lipase-catalyzed trans-esterification under supercritical conditions. Biochem Eng J, 29, 27–34.
127. Liu, S., Zhang, C., Hong, P. & Ji, H. (2006). Concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) of tuna oil by urea complexation: Optimization of process parameters. J Food Eng, 73, 203–209.
128. Smith, A. E. (1952). Crystal Structure of the Urea-Hydrogen Complexes. Acta Cryst, 5, 224-235.
129. Schlenk, H. & Holman, R.T. (1950). Separation and stabilization of FAs by urea complexes. J Am Oil Chem Soc, 27, 5001-5004.
130. Yoshikazu, T., Hirosi, Y., Kazuko, H. & Satoru, K. (1997). Method for refining and manufacturing fats and oils containing polyunsaturated FAs. European Patent 0773283.
131. FDA GRAS Notification (2002). GRAS Notice 000105: eicosapentaenoic and docosahexaenoic acid concentrates. www.accessdata.fda.gov/scripts/fcn/gras_notices/211666A.PDF (retrieved on May 2011)
132. FDA GRAS Notification (2006). GRAS Notice 000200. Eicosapentaenoic and docosahexaenoic acid concentrates. www.accessdata.fda.gov/scripts/fcn/gras_notices/701521A.PDF (retrieved on May 2011).
133. Lawson, L. D. & Hughes, B. G. (1988). Human absorption of fish oil FAs as triacylglycerols free FAs or ethyl esters. Biochem Biophys Res Communications, 152, 328–335.
134. Boyd, L. C., King, M. F. & Sheldon, B. (1992). A rapid method for determining the oxidation of n−3 FAs. J Am Oil Chem Soc 69, 4, 325–330.
135. Wijesundera, C., Ceccato, C., Watkins, P., Fagan, P., Fraser, B., Thienthong, N. & Perlmutter, P. (2008). Docosahexaenoic acid is more stable to oxidation when located at the sn−2 position of triacylglycerol compared to sn−1(3). J Am Oil Chem Soc, 85, 6, 543–548.

136. Mu, H. & Porsgaard, T. (2005). The metabolism of structured triacylglycerols. Progress in Lipid Research, 44, 430-48.
137. Hita, E., Robles, A., Camacho, B., González, P. A., Esteban, L., Jiménez, M. J., Muñío, M. M. & Molina, E. (2009). Production of structured triacylglycerols by acidolysis catalyzed by lipases immobilized in a packed bed reactor. Biochemical Engineering Journal, 46, 257-64.
138. Bottino, N. R., Vandenburg, G. A. & Reiser, R. (1967). Resistance of certain long-chain polyunsaturated fatty acids of marine oils to pancreatic lipase hydrolysis. Lipids, 2, 489-93.
139. Christensen, M. S., Hoy, C. E., Becker, C. C. & Redgrave, T. G. (1995). Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA) and decanoic acids: dependence on intramolecular triacylglycerol structure. American Journal of Clinical Nutrition, 61, 56-61.
140. Mu, H. (2006). Absorption of structured lipids and their applications in the diet. Lipid Technology, 12, 271-74.
141. Torres, C. F., Lin, B., Moeljadi, M., Hill Jr, D. G. (2003). Lipase-catalyzed synthesis of designer acyl-glycerols rich in residues of eicosapentaenoic, docosahexaenoic, conjugated linoleic, and/or stearic acids. European Journal of Lipid Science and Technology, 105, 614-23.
142. Iwasaki, Y. & Yamane, T. (2000). Enzymatic synthesis of structured lipids. Journal of Molecular Catalysis B: Enzymatic, 10, 129-40.
143. González Moreno, P. A., Robles Medina, A., Camacho Rubio, F., Camacho Páez, B. & Molina Grima, E. (2004). Production of structured lipids by acidolysis of an EPA- enriched fish oil and caprylic acid in a packed bed reactor: analysis of three different operation modes. Biotechnology Progress, 20, 1044-52.
144. Irimescu, R., Furihata, K., Hata, K., Iwasaki, Y. & Yamane, T. (2001). Utilization of reaction medium-dependent regiospecificity of *Candida antarctica* lipase (Novozym 435) for the synthesis of 1,3-dicapryloyl-2-docosahexanoyl (or eicosapentaenoyl) glycerol. Journal of the American Oil Chemists' Society, 78, 285-90.
145. Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition, 71, 171-75.
146. Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. American Journal of Clinical Nutrition, 70, 560-69.
147. Xu, X. (2000). Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. European Journal of Lipid Science and Technology, 287-303.
148. Houde, A., Kademi, A., Leblanc, D. (2003). Lipases and Their Industrial Applications. *An Overview*. Applied Biochemistry and Biotechnology, 118, 155-70.

149. Hita, E., Robles, A., Camacho, B., Ramírez, A., Esteban, L., Jiménez, M. J., Muñío, M. M., González, P. A., Molina, E. (2007). Production of structured triacylglycerols (STAG) rich in docosahexaenoic acid (DHA) in position 2 by acidolysis of tuna oil catalyzed by lipases. Process Biochemistry, 42, 415-22.

150. Xu, X., Porsgaard, T., Zhang, H., Adler-Nissen, J., Høy, C.E. (2002). Production of structured lipids in a packed-bed reactor with *Thermomyces lanuginose* lipase. Journal of the American Oil Chemists´ Society, 79, 561-65.

151. Shimada, Y., Sugihara, A., Maruyama, K., Nagao, T., Nakayama, S., Nakanno, H., Tominaga, Y. (1996). Production of Structured Lipid Containing Docosahexaenoic and Caprylic Acids Using Immobilized *Rhizopus delemar* Lipase. Journal of Fermentation and Bioengineering, 81, 299-303.

152. Zhou, D., Xu, X., Mu, H., Høy, C.E., Adler-Nissen, J. (2000). Lipase-catalyzed production of structured lipids via acidolysis of fish oil with caprylic acid. Journal of Food Lipids, 7, 263-74.

153. Schmid, U., Bornscheuer, U.T., Soumanou, M.M., McNeill, G.P., Schmid, R.D. (1998). Optimization of the Reaction Conditions in the Lipase-Catalyzed Synthesis of Structured Triglycerides. Journal of the American Oil Chemists´ Society, 75, 1527-31.

154. Muñío, M. M., Esteban, L., Robles, A., Hita, E., Jiménez, M. J., P.A. González, Camacho, B., Molina, E. (2008). Synthesis of 2-monoacylglycerols rich in polyunsaturated fatty acids by ethanolysis of fish oil catalyzed by 1,3 specific lipases. Process Biochemistry, 43, 1033-39.

155. Muñío, M. M., Robles, A., Esteban, L., González, P. A., Molina, E. (2009). Synthesis of structured lipids by two enzymatic steps: Ethanolysis of fish oils and esterification of 2-monoacylglycerols. Process Biochemistry, 44, 723-30.

156. Irimescu, R., Furihata, K., Hata, K., Iwasaki, Y., Yamane, T. (2001). Two-Step Enzymatic Synthesis of Docosahexaenoic Acid-Rich Symmetrically Structured Triacylglycerols *via* 2-Monoacylglycerols. Journal of the American Oil Chemists´ Society, 78, 743-48.

157. Senanayake, S. P. J. N. & Shahidi, F. (2001). Modified Oils Containing Highly Unsaturated Fatty Acids and Their Stability. In F. Shahidi and J.W. Finley (Eds.), Omega-3 Fatty Acids: Chemistry, Nutrition and Health Effects. American Chemical Society Symposium Series 788 (pp. 162–173). Washington, DC: American Chemical Society.

158. Shahidi, F. & Wanasundara U.N. (1997). Measurement of Lipid Oxidation and Evaluation of Antioxidant Activity. In F. Shahidi (Ed.), Natural Antioxidants: Chemistry, Health Effects, and Application (pp. 379–395.) Champaign, IL: AOCS Press.

159. Gray, J.I. (1978). Measurement of Lipid Oxidation: A Review. Journal of the American Oil Chemists' Society, 55, 539–546.

160. Namal Senanayake, S. P. J. & Shahidi. F. (2002). Oxidative Stability of Structured Lipids Produced from Borage (*Borago officinalis* L.) and Evening Primrose (*Oenothera biennis* L.) Oils with Docosahexaenoic Acid. Journal of the American Oil Chemical Society, 79, 1003-1013.

161. Guil-Guerrero, J. L., Giménez-Giménez, A., Robles-Medina, A., Rebolloso-Fuentes, M. M., Belarbi, H., Esteban-Cerdán, L. & Molina-Grima, E. (2001). Hexane reduces peroxidation of fatty acids during storage. European Journal of Lipid Science and Technology, 103, 271–278.

162. Hamam, F. & Shahid, F. (2004). Synthesis of Structured Lipids via Acidolysis of Docosahexaenoic Acid Single Cell Oil (DHASCO) with Capric Acid. Journal of Agricultural and Food Chemistry, 52, 2900-2906.

163. Maduko, C. O., Park, Y. W. & Akoh, C. C. (2008). Characterization and Oxidative Stability of Structured Lipids: Infant Milk Fat Analog. Journal of the American Oil Chemists' Society, 85, 197-204.

164. Albert, C. M., Oh, K., Whang, W., Manson, J. E., Chae, C., Stampfer, M. J., Willet, W. C. & Hu, F. (2005). Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. Circulation, 112, 3232-38.

165. Barrow, C. J., Nolan, C. & Holub, B. J. (2009). Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. Journal of Functional Foods, 1, 38-43.

166. Kurowska, E. M., Dresser, G. K., Deutsch, L., Vachon, D. & Khalil, W. (2003). Bioavailability of omega-3 essential fatty acids from perilla seed oil. Prostaglandins, Leukotrienes and Essential Fatty Acids, 68, 207–12.

167. Shidhu, S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology, 38, 336-44.

168. Wijendran, V. & Hayes, C. (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annual Review of Nutrition, 24, 597-615.

169. Burr, M. L., Fehily, M., Rogers, S., Welsby, E., King, S. & Sandham, S. (1989). Diet and reinfarction trial (DART): design, recruitment, and compliance. European Heart Journal, 10, 558-67.

170. GISSI - Prevenzione Investigators (1999). Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E in 11,324 patients with myocardial infarction: results of the GISSI. Prevenzione trial. Lancet, 354, 447–55.

171. Morris, M. C., Sacks, F. & Rosner, B. (1993). Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation, 88, 523-33.

172. Bordin, P., Bodamer, O. A., Venkatesan, S., Gary, R. M., Bannister, P. A. & Halliday, D. (1998). Effects of fish oil supplementation on apoliprotein B 100 production and lipoprotein metabolism in normolipidaemic males. Eur. J. Clin. Nutr., 52(2), 104.
173. Willunsen, N., Vaagenes, H., Lie, O., Ustan, A. C. & Berge, R. G. (1996). Eicosapentaenoic acid, but not docosahexaenoic acid, increases mitochondrial fatty acid oxidation and upregulates 2,4-dienoyl- CoA reductase gene expression in rats. Lipids, 31(6), 579.
174. Halminski, M. A., Marsh, J. B. & Harrison, E. H. (1991). Comparative effects of dietary fish oil (FO), safflower oil (SO) and palm oil (PO) on selected enzymes of hepatic lipid metabolism. In Simopoulos, A.P., Kiefer, R.R., Martin, R.E., Barlow, S.M. (Ed.), Textbook of Health effects of ω-3 polyunsaturated fatty acids in seafood (552). World Rev. Nutr. Diet. Basel, Karger.
175. Roberts, C. K & Sindhu, K. K. (2009). Oxidative stress and metabolic syndrome. Life Sciences, 84, 705-12.
176. Mertens, A, & Holvoet, P. (2001). Oxidized LDL and HDL: antagonists in atherothrombosis. FASEB Journal, 15, 2073-84.
177. Griffin, B. A., Minihane, A.M., Furlonger, N., Chapman, C., Murphy, M., Willians, D., Wright, J. J. & Williams, C. M. (1999). Inter-relationships between small, dense low-density lipoprotein (LDL), plasma triacylglycerol and LDL apoprotein B in an atherogenic lipoprotein phenotype in free-living subjects. Clinical Science, 97, 269-76.
178. Steinberg, D. (1997). Low density lipoprotein oxidation and its pathobiological significance. The Journal of Biological Chemistry, 272, 20963-966.
179. Blonk, M. C., Bilo, H. G., Nauta, J. P, Popp-Snijders, C., Mulder, C. & Donker, A. M. (1990). Dose-response effects of fish-oil supplementation in healthy volunteers. Am J Clin Nutr, 52, 120-27.
180. Sanders, T. A. B., Oakley, F. R., Miller, G. J., Mitropoulos, K. A., Crook, D. & Oliver, M. F. (1997). Influence of n-6 versus n-3 polyunsaturated fatty acids in diets low in saturated acids on plasma lipoproteins and hemostatic factors. Arteriosclerosis, Thrombosis, and Vascular Biology, 17, 3449-460.
181. Mori, T. A., Burke, V., Puddey, I. B., Watts, G. F., O´Neal, D. N., Best, J. D. & Beilin, L. J. (2000). Purified eicosapentaenoic and docosahexaenoic acids have different effects on serum lipids and lipoproteins, LDL particles size, glucose, and insulin in mildly hyperlipidemic men. Am J Clin Nutr, 71, 1084-94.
182. Gerasimova, E., Perova, N., Ozerova, I., Polessky, V., Metelskaya, V., Sherbakova, I., Lavachev, M., Kulakova, S., Nikitin, Y. & Astakhova, T (1991). The effect of dietary polyunsaturated fatty acids on HDL cholesterol in Chukot residents vs Muscovites. Lipids, 26(4), 261.

183. Wilkinson, P., Leach, C., Ah-Sing, E. E., Hussain, N., Miller, G. J., Millward, D. J. & Griffin, B. A. (2005). Influence of α-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an therogenic lipoprotein phenotype. Atherosclerosis, 115-24.

184. Satoh, N., Shimatsu, A., Kotani, K., Skane, N., Yamada, K, Suganami, T., Kusuya, H. & Ogawa, Y. (2007). Purified eicosapentaenoic acid reduces small dense LDL; remnant lipoprotein particles, and C-rective protein in metabolic syndrome, 30,144-46.

185. Xia, P., Vadas, M. A., Rye, K-A, Barter, P. J. & Gamble, J. R. (1999). High density lipoproteins (HDL) interrupt the sphingosine kinase signaling pathway. The Journal of Biological Chemistry, 274, 33143-147.

186. Sethi, A. A., Sampson, M., Warnick, R, Muniz, N., Vaisman, B., Nordestgaard, B. G., Tybjaerg-Hansen, A. & Remaley, A. T. (2010). High Pre-β1 HDL concentrations and low lecithin: cholesterol acyltransferase are strong positive risk markers for ischemic heart disease and independent of HDL-Cholesterol. Clinical Chemistry, 56, 1128-137.

187. Harris, W.S., Lu, G., Rambjør, G.S, Wålen, A.I., Ontko, J.A., Cheng, Q. E & Windsor, S.H. (1997). Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. Am. J. Clin. Nutr., 66, 254

188. Badellino, K. O., Wolfe, M. L., reilly, M. P. & Rader, D. J. (2006). Endothelial lipase concentrations are increased in metabolic syndrome and associated with coronary atherosclerosis. PLoS.Medicine, 3, 245-52.

189. Yasuda, T., Ishida, T, & Rader, D. J. (2010). Update on the role of endothelial lipase in high-density lipoprotein metabolism, reverse cholesterol transport and atherosclerosis. Circulation Journal, 74, 2263-70

190. Chen, S & Subbaiah, P. V. (2007). Phospholipid and fatty acid specificity of endothelial lipase: potential role of the enzyme in the delivery of docosahexaenoic acid (DHA) to tissues. Biochim Biophys Acta, 1771, 1319-28.

191. Shiu, S. W. M., Tan, K. C. B., Huang, Y., & Wong, Y. (2008). Type 2 diabetes mellitus and endothelial lipase. Atherosclerosis, 198, 441-47.

192. Huang, J., Qian, H-Y, Li, Z-Z, Zhang, J-M, Wang, S., Tao, Y., Gao, Y-L, Yin, C-Q; Que, B., Sun, T., Zhao, Z-Y & Li, Z. (2010). Role of endothelial lipase in atherosclerosis. Translational Research, 156, 1-6.

193. Klein-Platat, C., Drai, J., Oujaa, M., Schlienger, J-L,.& Simon, C. (2005). Plasma fatty acids composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. Am J Clin Nutr, 82, 1178-84.

194. Barter, P. J., Ballantyne, C. M., Carmena, R., Castro Cabezas, M., Chapman, M. J., Couture, P., de Graaf , J., Durrington, P.N., Faergeman, O., Frohlich, J., Furberg, C. D., Gagne, C., Haffner, S. M., Humphries, S. E., Jungner, I., Krauss, R. M., Kwiterovich, P.,

Marcovina, S., Packard, C. J., Pearson, T. A., Reddy, K. S., Rosenson, R., Sarrafzadegan, N., Sniderman, A. D., Stalenhoef, A. F., Stein, E., Talmud, P. J., Tonkin, A. M., Walldius, G., Williams, K. M. (2006). Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. Journal of Internal Medicine; 259, 247-58.

195. Doughman, S. D., Srirama Krupanidhi, S. and Sanjeevi, C. B. (2007). Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Current Diabetes Reviews, 3, 198-203.

196. Hooper, L., Thompson, R. I. & Harrison, R. A. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ, 332, 752-755.

197. Das, U. (2000). Beneficial effects of n-3 fatty acids in cardiovascular diseases: but, why and how?, Prostaglandins, Leukotrienes and Essential Fatty Acids, 63, 351-62.

198. Engler, M. M., Engler, M. B. & Kroetz, D. L. (1999). The effects of a diet rich in docosahexaenoic acid on organ and vascular fatty acid composition in spontaneously hypertensive rats. Prostaglandins, Leukotrienes and Essential Fatty Acids, 61, 289-95.

199. Harris W. & Schacky C. (2004). The Omega-3 Index: a new risk factor for death from coronary heart disease?. Preventive Medicine, 39, 212– 20.

200. Morris, M. C., Sacks, F. & Rosner, B. (1993). Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation, 88, 523-33.

201. Nordoy, A., Hatcher, L. F., Ullmann, D. L. & Connor, W. E. (1993). Individual effects of dietary saturated fatty acids and fish oil on plasma lipids and lipoproteins in normal men. American Journal of Clinical Nutrition, 57, 634-39.

202. Thies, F., Garry, J. M., Yaqoob, P., Rerkasem, K., Williams, J., Shearman, C. P., Gallagher, P.J., Calder, P. C. & Grimble, R. F. (2003). Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomized controlled trial. Lancet, 361, 477-85.

203. Mazza, M., Pomponi, M. & Janiri, L. (2007). Omega-3 fatty acids and antioxidants in neurological and psychiatric diseases: An overview. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 31, 12-26.

204. Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. J. Am. Coll. Nutr., 21, 495-505.

205. Schmitz, G. & Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. Progress in Lipid Research, 47, 147–51.

206. Dyall, S. C. & Michael-Titus, A. T. (2008). Neurological benefits of omega-3 fatty acids. Neuromolecular Medicine, 4, 219-35.

207. Kimura, F., Endo, Y., Fujimoto, K., Doisaki, N. & Koriyama, T. (2005). Administration of two oils rich in n-3 long-chain polyunsaturated fatty acids to rat pups of dams fed a diet high in fat and low in n-3 polyunsaturated fatty acids. Fisheries Science, 71, 431–40.
208. Ward, O. & Singh, A. (2005). Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry, 40, 3627–52.
209. Pegorier, S., Wagner, L. A., Gleich, G. J. & Pretolani, M. (2006). Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells, Journal of Immunology, 177: 4861–4869.
210. Risé, P., Eligini, S., Ghezzi, S., Colli, S. & Galli, C. (2007). Fatty acid composition of plasma, blood cells and whole blood: Relevance for the assessment of the fatty acid status in humans. Prostaglandins, Leukotrienes and Essential Fatty Acids, 76: 363-69.
211. Russo, C., Olivieri, O., Girelli, D., Guarini, P., Pasqualini, R., Azzini, M. & Corrocher, R. (1997). Increased membrane ratios of metabolite to precursor fatty acids in essential hypertension. Hypertension, 29: 1058-63.
212. Harris, W.S. (2008). The omega-3 index as a risk factor for coronary heart disease. Am J Clinical Nutrition, 87 (6): 1997S-2002S.
213. Shapiro, H., Tehilla, M., Attal-Singer, J., Bruck, R. & Luzzatti, R. (2010). The therapeutic potential of long-chain omega-3 fatty acids in non-alcoholic fatty liver disease. Clinical Nutrition, 30, 6-19.
214. Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 75, 197-202.
215. Garofolo, A. & Petrilli, S. (2006). Omega-3 and 6 fatty acids balance in inflammatory response in patients with cancer and cachexia. Rev. Nutr. Campinas, 19, 611-21.
216. He, X., Yang, X. & Guo, Y. (2007). Effects of different dietary oil sources on immune function in cyclophosphamide immunosuppressed chickens. Animal Feed Science and Technnology, 139, 186-200.
217. Medina, A. R., Cerdán, L. E., Giménez, A. G., Páez, B. C., González, M. J. I. & Grima, E. M. (1999). Lipase-catalyzed esterification of glycerol and polyunsaturated fatty acids from fish and microalgae oils. Journal of Biotechnology, 70, 379-91.
218. Moyad, M. (2005). An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: Part I; Urologic Oncology: Seminars and Original Investigations, 23, 28-35.
219. Chapkin, R. S., Davidson, L. A., Ly, L., Weeks, B. R & Lupton, J. (2007). Immunomodulatory effects of (n-3) fatty acids: putative link to inflamatio and colon cancer. The Journal of Nutrition, 200S-203S.
220. Liblau, R. S., Singer, S. M. & McDevitt, H. O. (1995). Th1 and th2 $CD4^+$ T cells in the pathogenesis of organ-specific autoimmune diseases. Immunology Today, 16, 34-38.

221. Abbas, A. K, Murphy, K. M. & Sher, A. (1996). Functional diversity of helper T lymphocytes. Nature, 383, 787-93.

222. Neurath, M. F., Finotto, S. & Glimcher, L. H. (2002). The role of Th1/Th2 polarization in mucosal immunity. Nature Medicine, 8, 567-73.

223. Zhang, P., Smith, R., Chapkin, R. S. & McMurray, D. N. (2005). Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. The Journal of Nutrition, 1745-51.

224. Krauss-Etschmann, S., Harti, D. & Rzehak, P. (2008). Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-β levels after fish oil supplementation of pregnant women. J Allergy Clin Immunol, 121, 464-70.

225. Shaikh, S. R. & Edidin, M. (2006). Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. Am J Clin Nutr, 8, 1277-89.

226. Switzer, K. C., Fan, Y-Y., Wang, N., McMurray, D. N. & Chapkin, R. S. (2004). Dietary polyunsaturated fatty acids promote activation-induced cell death in Th1-polarized murine $CD4^+$ T-cells. Journal of Lipid Research, 45, 1482-92.

227. Fan, Y-Y., Ly, L. H., Barhoumi, R., McMurray, D. N. & Chapkin, R. S. (2004). Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. Journal Immunol, 173, 6151-60.

228. Zeyda, M., Watchaust, W. & Stulnig, T. M. (2003). Suppression on T cell signalling by polyunsaturated fatty acids: selectivity in inhibition of mitogen-activated protein Kinase and nuclear factor activation. Journal Immunol, 170, 6033-39.

229. Muppidi, J. R., Tschopp, J. & Siegel, R. M. (2004). Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. Immunity, 21, 461-65.

230. WHO / FAO, 2003. World Health Organization/Food and Agriculture Organization (2003). Diet, nutrition and the prevention of chronic diseases. *Report of a joint WHO/FAO expert consultation.* Genebra, WHO/FAO, WHO Technical Report Series 916.

231. ISSFAL, 2004. ISSFAL International Society for the Study of Fatty Acids and Lipids (2004) Recommendations for intake of polyunsaturated fatty acids in healthy adults, p 22

232. Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., Franklin, B., Kris-Etherton, P., Harris, W. S., Howard, B., Karanja N., Lefevre M., Rudel L., Sacks F., Van Horn L., Winston M., Wylie-Rosett J. (2006) Diet and lifestyle recommendations revisions. A scientific statement from the American Heart Association Nutrition Committee. Circulation 114, 82-96.

233. Kris-Etherthon PM, Harris WS & Appel LJ (2002). Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Circulation.; 106,2747-57. [Erratum, *Circulation*. 2003; 107:512.]
234. Conquer, J. and Holub, B. (1996). Supplementation with an Algae Source of Docosahexaenoic Acid Increases (n-3) Fatty Acid Status and Alters Selected Risk Factors for Heart Disease inVegetarian Subjects. Human and Clinical Nutrition, 126, 3032-3039.
235. Hooper L., Thompson R. I. &Harrison R. A. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ , 332, 752-755.
236. Schmitz, H. H., Poor, C. L., Wellman, R. B. & Erdman, J. W., Jr (1991) Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. J. Nutr. 121,1613-1621.
237. Puhakainen, I., Ahola, I. & Yri-Järvinen, H. (1995). Dietary supplementation with n-3 fatty acids increases gluconeogenesis from glycerol but not hepatic glucose production in patients with non-insulin-dependent diabetes mellitus. Am J Clin Nutr, 61, 121-26.

View publication stats