Abstract:

Title: PROCESS FOR THE PURIFICATION OF OILS

The invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material, especially squid visceral material, said process comprising: processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction; optionally refining one or both of the lipid fractions thus obtained; and recombining the two fractions, or portions thereof, to produce a mixture having the desired characteristics. In certain embodiments, the sample is processed using a polar solvent, preferably ethanol, as an entrainer in a supercritical fluid extraction procedure, preferably using carbon dioxide, to provide both neutral and polar lipid fractions.
Process for the purification of oils

The present invention relates to processes for the purification of extracts, especially oils, from marine animal visceral parts, especially those comprising squid liver, which are suitable for consumption and also to extracts producible by said processes. The extracts of the invention are high in omega 3 polyunsaturated fatty acids, in particular high in docosahexaenoic acid (DHA) and have a high phospholipid content. Furthermore, the extracts of the invention are low in components such as cholesterol and docosapentaenoic acid (DPA) as well as being low in environmental contaminants. The present invention also relates to the nutraceutical and pharmaceutical uses of said oils.

The disclosure of each reference set forth herein is incorporated by reference in its entirety.

The health benefits of consuming long-chain omega 3 fatty acids are well established. Dietary supplementation of omega 3 fatty acids has been linked with a reduced risk of coronary heart disease, ischemic and thrombotic stroke as well as some cancers. Certain mental disorders, such as aggression and schizophrenia, may be ameliorated by omega 3 supplements.

Long-chain polyunsaturated fatty acids represent an important food supplement. Mammals lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10 and hence certain fatty acids (e.g. linoleic acid) are essential supplements for humans. In the body, essential fatty acids are primarily used to produce substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury or infection.

References herein to fatty acids are intended to cover fatty acid derivatives, such as salts and esters as well as glycerides (e.g. triglycerides) and phospholipids thereof.

An important sub-class of polyunsaturated fatty acids are the omega 3 fatty acids, which all have a carbon-carbon double bond in the omega 3 position, i.e. the third carbon-carbon bond from the terminal methyl end (ω) of the carbon chain. The nutritionally important omega 3 fatty acids include α-linolenic acid (ALA) as well as eicosapentaenoic acid (EPA) and DHA.
Omega 3 fatty acids are available from a number of natural sources. These include higher animals, especially fish such as cod and salmon and mammals such as seals which are rich in DHA and EPA. Squid is a particularly rich source of DHA and EPA as well as other polyunsaturated fatty acids. DHA is also found in algae such as *Ciypthecodinium cohnii*.

Omega 3 products are typically evaluated on their total content of omega 3 fatty acids. Lately there has been a growing interest in providing either a high EPA and low DHA product or *vice versa*, since some of the beneficial effects of omega 3 have been ascribed to EPA whereas others are ascribed to DHA. Very recently, the distinctive effects of another omega 3 fatty acid, docosapentaenoic acid (DPA), have been reported. In one study, DPA was shown to be a potent stimulator of endothelial cell migration *in vitro*. The potency of DPA was tenfold higher than that of EPA. Another recent study has suggested that DPA might interfere more strongly with the cyclo-oxygenase pathways than EPA or DHA and thereby accelerate the lipoxygenase pathway, thus more strongly inhibiting platelet aggregation. Although both of these effects may have positive effects on cardiac health, they may not be beneficial for other medical indications. For example, Leucotriene B4 (for which arachidonic acid (ARA) is the precursor) is known to be a potent pro-inflammatory eicosanoid in asthma related diseases. If the lipoxygenase pathway is up-regulated by DPA (as is suggested by the above-mentioned studies), the present inventors consider that asthmatic patients would benefit from an omega 3 product with as low a content of DPA as possible. The omega 3 concentrates of the invention will therefore be of benefit to people that suffer from problems associated with too high a production of eicosanoids, for example catalyzed by lipoxygenase.

DPA is present in some marine oils in levels of greater than 50% of the DHA content of the oils. Therefore, not only does DPA represent an obstacle for producing high DHA content concentrates by distillation, but it is also present in the pharmaceutical and nutraceutical supplements presently available on the market.

The research on the possible health effects of the individual omega 3 fatty acids is still in its infancy and the two studies described above are believed to be the only published data regarding DPA. Nevertheless, more data are expected to be published since pure forms of DPA have recently become available for research in quantities allowing animal models to be
used. Therefore it is of interest to develop technologies for provision of omega 3 concentrates that contain more pure forms of omega 3 (e.g. with and without DPA).

Omega 3 concentrates for consumption should preferably be low in arachidonic acid (ARA) since this is a precursor of several pro-inflammatory eicosanoids. Certain fish oils are available that do contain a high level of EPA (analogues of which, as precursors, are typically less inflammatory) and a low level of ARA. These oils are useful raw materials for achieving a high concentrate of EPA during the purification and concentration process.

Phospholipids are generally amphipathic lipids which comprise the group:

\[
\text{RO} \quad \text{P} \quad \text{O} \quad \text{X} \quad \text{R}
\]

wherein R is a group usually carrying two fatty acyl side-chains, which may be the same or different, and wherein X is a polar group such as choline or ethanolamine. Examples of phospholipids are phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl serine.

Recent studies into the effects of omega 3-containing phospholipids suggest that administration of omega 3 fatty acids in phospholipids is significantly more effective than administration of the omega 3 fatty acids in other forms, such as triglycerides. Conditions which respond better to administration of phospholipids include attention deficit/hyperactivity disorder (ADHD) and premenstrual syndrome/dysmenorrhoea. It may be that phospholipids enjoy a greater bioavailability and/or are more absorbable across biological membranes than other lipid forms. It is therefore postulated that compositions comprising omega 3 fatty acid phospholipids will become extremely desirable in the future.

Oils with a high level of DHA (above approximately 20%) which also contain a high level of phospholipids are presently not available in the market for human consumption. The only food-grade omega 3 product with a high level of phospholipids is krill oil, but this oil only contains around 6% DHA. Squid is known to contain a very high level of omega 3 fatty acids and squid by-products have been used to produce oils for use as additives in aquaculture feeds, especially for shrimp production. This squid oil is very dark in colour, almost black, is essentially free of phospholipids, and contains high levels of free fatty acids
and cholesterol. Squid oil for human consumption has recently appeared on the market, but this oil is extracted in such a way that essentially all of the phospholipid component is lost.

The squid liver contains extremely active lipases and the development of free fatty acids in the liver begins as soon as the animal is caught. Due to this problem and also due to its high cholesterol content, little interest has been paid to squid liver as a raw material for products designed for human consumption. Heavy metal contamination in the squid visceral parts has also been a matter of concern. In contrast, fish oils, especially fish liver oils typically consist mainly of triacyl glycerols and as such they can be processed in many different ways to yield a pure, light-coloured, transparent and good tasting oil with low content of cholesterol and environmental contaminants.

Refining of fish oils and vegetable oils typically includes, among other steps, bleaching and deodorization. Prior to any bleaching process, a degumming or washing step is generally necessary which removes most of the phospholipids. The bleaching process itself removes most of the residual phospholipids and soap (if alkali refining has been used) which is a prerequisite for obtaining a light oil after the deodorization process. Bleaching processes will also typically reduce the content of certain environmental contaminants (such as pesticides and/or arsenic compounds) that might be found in the source oil. Adsorption processes may also be used to reduce the levels of contaminants in the oil. Molecular distillation might also be necessary in place of, or in addition to, adsorption processes.

Omega 3 fatty acids can also be found at high levels in some marine phospholipids, but when processed the conventional way, phospholipids are lost in the degumming or alkali refining steps. Currently, the only omega 3 product containing high levels of phospholipids on the market for supplements and food applications are derived from krill. Krill oil from the main producer is produced by extraction of lipids with acetone and ethanol and, after evaporation of the solvents, the oil is provided in soft gelatin capsules. Typically those products are rather low in omega 3 fatty acids, especially low in DHA, and the cholesterol level is relatively high at around 1 to 2%.

A recent study prepared ethyl esters from squid liver oil and showed that levels of up to 43% DHA could be achieved by molecular distillation. This product did not contain any phospholipids since these are lost when preparing ethyl esters by the method described.
The phospholipid fraction of the lipids of squid liver is, as also seen for other marine phospholipids, high in DHA compared to DPA. However, it has not previously been recognised that the unusually high ratio of DHA to DPA in squid oil, e.g. in the neutral fractions of the oil, may be utilised to prepare concentrates for consumption. The content of DHA in the total squid oil might be as high as 30% (weight calculated as free fatty acid per weight of oil) but, more importantly, the DPA may be as low as less than 1%. The omega 3 product obtained from the raw material and the process herein described will give an omega 3 oil with an exceptionally high content of DHA compared to DPA.

Most studies into the dietary effects of omega 3 fatty acids have used fish oils as the source of omega 3 fatty acids, such oils being the prevailing source for extraction and purification of Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) acids. Omega 3 concentrates for consumption are typically made from fish oils by molecular distillation; EPA concentrates of up to 45-50% purity and DHA concentrates of 50-55% purity can be produced if the raw materials are carefully selected. If concentrates with higher omega 3 concentrations are desired, additional techniques, such as the use of enzymes or urea complexation both currently in industrial use, may be employed. The highest purities are conventionally achieved by adding a final purification step involving some kind of chromatography (flash chromatography or supercritical fluid chromatography).

In the process of concentrating EPA and DHA, it is necessary to obtain a source containing a high concentration of EPA and DHA. To be able to distil omega 3 fatty acids, they have to be separated into the free fatty acid form (e.g. detached from the glycerol backbone if they are present as triglycerides) and the prevailing technology is to trans-esterify the fatty acids into ethyl esters. Conventional distillation with a theoretical high plate number apparatus cannot be used because highly unsaturated fatty acids undergo isomerisation (e.g. into trans configurations) and/or conjugation of their double bonds when kept at temperatures above approximately 180-190°C for prolonged periods. As a result, distillations by short path distillations or molecular distillations in which the product is evaporated from a thin film surface at a very high vacuum are typically performed. Typically, the oil is in contact with the hot surface for less than 60 seconds. The drawback with these types of distillations is their relatively low theoretical plate number which leads to a rather poor separation power. The concentration that can be achieved is thus highly dependent on the composition of the
raw material. The ethyl esters of EPA and DHA do not have very different vapour pressures, even though they differ in chain length (20 carbons for EPA and 22 for DHA). DHA concentrates will therefore always contain a certain amount of EPA and vice versa if both fatty acids are present in the raw material. However, the most problematic fatty acids (those which prevent a high concentration by distillation) are those having the same chain length as the desired product, such as EPA or DHA. In marine oils these are typically C 22:5 (DPA) and C 22:1, both having a chain length equal to DHA, and C 20:4 n-6 (ARA) and C 20:1, having the chain length of EPA. Preferred raw materials for distillation contain a very low level of these fatty acids. The content of these "problematic" fatty acids depends on the source of the marine oil. The fatty acid profile in the fish reflects the fatty acid profile of the prey of the fish and, typically, the monounsaturated long chain acids (C 20:1 and C 22:1) are prominent in fish harvested in the northern hemisphere whereas several fish species in the cold waters of the southern hemisphere are almost devoid of those long chain mono-enes. Hence oils from fish caught off the coast of Chile and Peru are currently the most valuable raw materials for production of distilled concentrates of EPA and DHA.

Long chain mono-enes may be reduced substantially if commercially available enzymes are used during the purification process. One such method for the reduction of long chain mono-enes involves the production of free fatty acids from the fish oil which is then contacted with immobilized esterifying enzymes to produce glycerides (glycerol being added to the mixture). Enzymes are chosen which esterify the free fatty acids of low unsaturation at a much higher rate than long chain polyunsaturated fatty acids. Indeed, some enzymes even discriminate fairly well between EPA and DHA in this respect. At a point during the reaction, free EPA and DHA show a peak in concentration in the mixture, whereas the less unsaturated fatty acids are esterified with glycerol and exists predominantly in mono-, di-, or triglyceride forms. The reaction is stopped at this point and the free fatty acids are distilled off. The process can be repeated to achieve a good separation between EPA and DHA.

Urea complexation technology is also used on an industrial scale to enhance omega 3 fatty acid concentration in extracts. Saturated fatty acids, particularly the very long chain fatty acids and also mono-, and di-, unsaturated fatty acids (though to a lesser extent), tend to form a complex with urea at reduced temperatures. The ethyl esters or free fatty acids are mixed with methanol (or ethanol) and urea. At temperatures of around 60°C the mixture is
homogenous and transparent and upon cooling the saturated fatty acids are precipitated as crystals and can be filtered off. The highly unsaturated fatty acids or ethyl esters remain in the solution and may be recovered.

Although enzymes and urea complexation can discriminate to some extent between similar acids, neither of these technologies enable clean separation of EPA from ARA or of DHA from DPA.

A goal of the present inventors was thus to provide compositions for consumption having a high level of DHA and a high level of phospholipids. Further objects were to provide a composition with a low DPA content compared to its DHA content, especially where the composition has a high level of DHA and a high level of phospholipids and has a low level of DPA and a low level of cholesterol. To this end, the inventors have developed processes for the preparation of such compositions and contemplate pharmaceutical and nutraceutical preparations prepared from said compositions.

Unless otherwise stated, all weights and percentage weights of fatty acids described herein refer to the weight of said fatty acid when calculated as a free fatty acid per weight of composition, e.g. per weight of oil.

The present inventors have found that marine animal visceral material, e.g. of anchovies, sardines, cod, salmon, seal and squid, especially squid visceral material, are particularly suitable for preparing compositions for consumption having a high level of DHA and a high level of phospholipids. Examples of squids that may be used to provide compositions according to the invention include Sepiola atlantica, Illex argentinus, Todarodes sagittatus, Gonatus sp. and Todarodes pacificus. The skilled person will readily appreciate that any source having the appropriate levels of fatty acids of interest may be amenable to use in the processes of the invention and to use for the preparation of compositions of the invention.

The present inventors have discovered that marine animal visceral parts, especially squid liver oil, or an oil made from visceral material of squid is a superior source of high level concentrates of DHA. Although it had previously been described that squid and squid oils contain the omega 3 fatty acids EPA and DHA, it was not known that squid is an excellent source for the production by distillation of very high concentrates of DHA having a very low concentration of DPA.
Table 1 below shows data obtained by batch analysis of raw marine oils available for omega 3 concentration. Values shown are area% by GC analysis. The corresponding % by weight (mg/100mg of oil) is slightly less:

<table>
<thead>
<tr>
<th></th>
<th>Seal oil</th>
<th>Chilean fish oil</th>
<th>Sepiola atlantica</th>
<th>Illex argentinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td>7.7</td>
<td>10.6</td>
<td>20.5</td>
<td>23.6</td>
</tr>
<tr>
<td>DPA</td>
<td>3.8</td>
<td>1.5</td>
<td>1.0</td>
<td>0.83</td>
</tr>
<tr>
<td>DHA:DPA ratio</td>
<td>2.0</td>
<td>7.1</td>
<td>20.5</td>
<td>28.5</td>
</tr>
</tbody>
</table>

As seen from Table 1, the two raw squid oils contain particularly high amounts of DHA compared to DPA (both over a 20:1 ratio of DHA:DPA). Without wishing to be bound by theory, the inventors postulate that elongase, responsible for converting EPA obtained through ingestion of prey animals into DPA, of squid shows a very low enzymatic activity. Alternatively, DPA produced in vivo from EPA or obtained via food directly is desaturated by delta-4 desaturase to a higher degree in squid than is seen fish and mammals. In either case, squid oil is a particular interesting source for the production of pure concentrates of DHA that contain little, or no, DPA.

Japanese patent application JP 2000-060432 discloses a method of extracting lipids from a meal processed from squid by-products. However, squid meals are very unstable and stability is often improved by use of antioxidants not approved for human consumption.

Liang, Jer-Hour et al. (J. Am. Oil Chem. Soc. 2000, Vol. 77, no. 7, pp. 773-777) describe the fractionation of squid visceral oil ethyl esters by short-path distillation. The oils produced by this method do not contain any phospholipids and no data is provided by the authors regarding the DPA content of the distilled fractions.

The inventors disclose herein a process for obtaining a high quality, natural, food-grade fatty acid-comprising extract from marine animal visceral material, e.g. from the liver of squid, which extract is very low in environmental contaminants. The desired oil may be produced by supercritical extraction of the marine animal visceral material, followed by a
dual-stream refining process, before the refined fractions are recombined. For example, extracting lipids from squid liver followed by one or more refining steps of the neutral part of the oil before this is mixed back into the polar fraction of the extract, provides the desired oil with a composition and purity not found in any oil on the market.

The process of the invention preferably comprises the steps of immediate freezing of the lipid-containing sample (e.g. the liver or visceral parts containing the liver); freeze drying of the frozen sample; supercritical extraction of lipids from the sample, preferably in two steps; refining of parts of the extracted lipids; and finally blending of the different fractions to produce the desired composition. Alternatively, the supercritical extraction of the freeze-dried sample can be done in one operation with a polar entrainer followed by a countercurrent supercritical separation of the oil into two fractions, one essentially polar and one essentially non-polar.

Thus, in a first aspect, the invention provides a process for producing a food-grade fatty-acid extract from a sample of squid visceral material, especially from squid liver, comprising:

a) processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction;

b) optionally refining one or both of the lipid fractions thus obtained; and

c) recombining the two fractions, or portions thereof, to produce a mixture having the desired characteristics.

By recombining "portions" of the non-polar and polar lipid fractions is meant mixing an amount, which may be between 0 and 100%, of each fraction to produce the mixture. The mixture preferably comprises greater than 50%, e.g. greater than 75% or 90% of the polar, i.e. the phospholipid containing, fraction. In one embodiment, the mixture comprises none of the non-polar fraction. In a preferred embodiment, at least part of the non-polar fraction is recombined with the polar fraction, or part thereof.

In place of squid, other suitable samples of marine animal visceral material could be used. Preferably the marine animal source is a non-exoskeletal animal source, for example an invertebrate fish source. Accordingly, in a further aspect, the invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material comprising:
a) processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction;
b) optionally refining one or both of the lipid fractions thus obtained; and
c) recombining the two fractions, or portions thereof, to produce a mixture having the desired characteristics. In a preferred embodiment, the marine animal is squid.

The preferred source of marine animal visceral material is, or comprises, squid liver. Preferably, the essentially polar lipid fraction comprises phospholipids.

To control the enzymatic activity, the lipid-containing sample is frozen as soon as possible after catching the marine animal. The frozen sample is then freeze dried after a relatively short time. The freeze dried material is kept at a low temperature, preferably below -20°C until the lipids are extracted with a solvent, e.g. after grinding of the material. Alternatively the sample is immediately processed after catching the marine animal by a procedure equivalent to that used in fish meal production. The dried meal is then extracted with a solvent as described.

Accordingly, in a preferred embodiment, the process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material (e.g. squid liver) further comprises the step or steps of immediate freezing and/or freeze drying of the marine animal visceral material before step (a). In another preferred embodiment, step (a) is carried out essentially immediately after the marine animal is caught.

Oils containing high levels of phospholipids are not suitable for undergoing conventional refining steps including alkali refinement to remove free fatty acids, or bleaching to remove pesticides to improve flavour and colour. Nor are such oils suitable for high temperature processing like molecular distillation for pesticide or cholesterol reduction due to their high viscosity and their tendency to foaming and browning in such processes.

The solution to this problem according to the invention is to split the oils into several fractions, to refine the several fractions separately, using appropriate technology, and then to combine the refined fractions. The inventors provide herein a detailed description of the process of refining a particularly difficult raw material, hitherto not used for human food applications.

The solvent extraction of the freeze dried sample or the dried meal may be performed in a two step procedure so as to split the lipid fractions into two product streams, an
essentially non-polar fraction (the non polar fraction) and an essentially polar fraction (the polar fraction). The non polar fraction can be extracted (i.e. obtained) by supercritical fluid extraction (SFE), for example with carbon dioxide or liquid nitrogen, followed by use of a polar solvent (for instance ethanol) for the extraction of the polar lipids. Alternatively, to obtain the polar fraction, ethanol or another polar solvent is used as an entrainer in a SFE procedure using carbon dioxide. Typically up to 20% by volume of ethanol would be necessary for removal of most of the polar lipids if carbon dioxide is the non polar solvent used. The skilled person would readily appreciate that different volumes will be necessary if alternative polar and non polar solvents are used.

Accordingly, in a preferred embodiment the invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material (e.g. squid liver) wherein step (a) is carried out in a two-step process comprising:

ai) supercritical fluid extraction of the sample, preferably using carbon dioxide, to obtain a lipid extract; and

15 a₂) treating the lipid extract of step (a₁) with a polar solvent, preferably ethanol, to enable the separation of an essentially neutral lipid fraction and an essentially polar lipid fraction.

In an alternative embodiment the step of processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction comprises the use of a polar solvent, preferably ethanol, as an entrainer in a supercritical fluid extraction procedure, preferably using carbon dioxide, to provide both neutral and a polar lipid fractions.

In an alternative method for obtaining multiple product streams, the visceral material is subjected to digestion, such as chemical digestion or enzymatic digestion e.g. with proteases, to digest the proteins and to liberate the lipid materials. In a typical example, fresh liver material or thawed freshly frozen material is minced and heated to around 60°C. The pH is then adjusted to the optimum value for the enzyme(s) chosen for digestion, the protease is added and reaction is allowed to take place, e.g. for approximately 1-2 hours under inert atmosphere. The digested material is allowed to segregate or is separated, e.g. using centrifugal forces, into separate products. Examples of products that may be separated by this process are:

- Neutral oil with little or no phospholipids;
A polypeptide-containing material comprising phospholipids; and
A polypeptide-containing material virtually free of phospholipids.

The polypeptide-containing materials, which may be obtained as solids, can be dried and used for various applications.

The polypeptide-containing material comprising the phospholipids may then be dried, and the phospholipid fraction extracted by conventional means, e.g. using a polar solvent, preferably ethanol. The phospholipids can also be extracted using supercritical extraction, e.g. with a polar entrainer added as described herein.

Examples of proteases that are suitable for use in the process described herein would be apparent to the skilled person and include Protex 6L (Genencor®) and Protamex® (Novozymes®). Operating and optimum conditions for protease digestion with any given enzyme are generally known in the art and typically include pH values from 2 to 9 and temperatures from 25 to 60°C.

Thus, in preferred embodiment, the invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material (e.g. squid liver) wherein step (a) is carried out in a two-step process comprising:

a1) subjecting the sample to enzymatic digestion by one or more proteases to obtain an essentially neutral lipid fraction and a fraction comprising peptide material and polar lipids; and

a2) extracting an essentially polar lipid fraction from the fraction comprising peptide material and polar lipids, optionally with or after drying of said fraction.

The first product stream provided in step (a), the non polar fraction which is essentially free of phospholipids, may then be treated by conventional methods available to remove any components that are not required, such as cholesterol, undesired (e.g. free) fatty acids and environmental contaminants. Cholesterol may be removed by molecular distillation. Environmental contaminants can be removed by distillation, optionally by adsorption technology (bleaching clay and/or activated charcoal or other adsorbents). The use of multiple separation vessels in the effluent stream of the supercritical fluid extraction vessel may also be used to reduce levels of cholesterol and free fatty acids. However, supercritical carbon dioxide technology alone is not a sufficient tool for obtaining oils virtually free of cholesterol.
Thus, in a preferred embodiment the invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material (e.g. squid liver) wherein step (b) comprises one or more of the following processes carried out on the essentially neutral fraction:

bi) distillation (e.g. molecular distillation) to remove cholesterol and environmental contaminants;

b₂) deodorisation; and

b₃) treatment with adsorbents such as bleaching clay or activated charcoal.

In a further preferred embodiment multiple separation vessels are used in collecting the effluent stream from the supercritical fluid extraction vessel.

The second product stream, the polar fraction should be obtained essentially free of cholesterol and many of the environmental contaminants. The polar fraction is obtained in diluted form in the entrainer (e.g. ethanol) in a SFE procedure. Preferably the only process step needed is to recover the oil, e.g. by evaporation of any solvent present under moderate heat and/or a reduced pressure. Due to the high viscosity of some polar lipids, it might be necessary to retain some solvent (e.g. around 5%) before the final blend is made. Any remaining solvent may then be removed from the final blend using conventional means.

If particular contaminants need to be removed from the polar fraction, this may be done before the solvent is removed, for example using flash chromatography or other separation and purification techniques known in the art.

Accordingly, in a preferred embodiment the invention provides a process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material (e.g. squid liver) wherein the step of refining one or more of the polar lipid fractions thus obtained (step (b) as described above) comprises one or more of the following processes carried out on the essentially polar fraction(s):

b₄) concentrating the polar fraction, e.g. by evaporation under reduced pressure and/or raised temperature; and

b₅) purifying the polar fraction, preferably using flash or column chromatography.

In another preferred embodiment, a polar solvent used to separate the polar fraction in step (a) is not fully removed from the polar fraction before the recombination of step (c) and
more preferably wherein the polar solvent is essentially removed following the recombination of the neutral and polar fractions.

The two product streams, the polar fraction and parts or all of the refined neutral fraction may then be combined to obtain an oil having the desired composition, in particular having the desired content of phospholipids. A final treatment, e.g. moderate heating under reduced pressure, may be necessary to remove the last traces of solvent in the final blend.

Thus, in a preferred embodiment of the process of invention, in step (c) part of the neutral fraction is added to the polar fraction to produce a mixture having the desired characteristics, e.g. a particular phospholipid content.

In a preferred embodiment, the fatty-acid extract produced by the process of the invention is formulated into a food-grade product, e.g. a nutraceutical product, for consumption, especially for human consumption. Preferably, the process according to the invention comprises a further step (d) of formulating the mixture produced in step (c) into said food-grade product.

A further aspect of the present invention therefore provides a food-grade fatty-acid extract, preferably a nutraceutical extract for human consumption, from a sample of marine animal visceral material (e.g. squid liver) produced by, or producible by, a process as described herein. Said extracts are encompassed with the term "composition" according to the invention.

Fatty acid extracts, or fatty acid-containing compositions, of the invention will typically contain (by weight of extract): less than 1%, preferably less than about 0.5% and especially preferably less than about 0.1% free fatty acids; more than about 15%, or 18%, preferably more than about 20% and more preferably more than about 30% DHA, e.g. in phospholipids; more than about 25%, preferably more than about 30% and especially more than about 40% phospholipids; and less than about 1.0%, preferably less than about 0.5%, more preferably less than about 0.2% cholesterol. It is preferred that extracts, or fatty acid-containing compositions, of the invention will comprise (by weight of extract): less than about 2%, preferably less than about 1.5%, especially preferably less than about 1% DPA; and will have a DHA:DPA ratio of greater than about 15:2, preferably greater than about 20:1, especially preferably greater than about 30:1.
Especially preferred are extracts having less than 0.5 % free fatty acids, more than 20% DHA, more than 30% phospholipids and less than 0.5% cholesterol. Particularly preferred are extracts having less than 0.1% free fatty acids, more than 30% DHA, more than 40% phospholipids and less than 0.5% cholesterol.

The compositions of the invention may comprise one or more oxidation inhibitors to delay the oxidation of the fatty acids. The composition may also be encapsulated, either micro-encapsulated or encapsulated in a larger scale, e.g. for direct administration in a capsule. Methods for encapsulating and micro-encapsulating fatty acids and fatty acid compositions are well known in the art.

The compositions for oral administration may typically be in the form of a liquid dosage (to be administered e.g. by the spoonful), a powder or tablet or a capsule. Powders, e.g. lyophilised formulations, may be prepared and optionally encapsulated according to methods known in the art. Capsules are particularly preferred. A bulk dosage form will typically consist of sufficient unit doses (e.g. capsules or tablets) to provide the required quantity of composition. The number and size of these unit doses will depend on the final dose required and also on the tolerances of the subject taking the unit doses. Preferably the unit dose for oral administration has a cylindrical or ellipsoidal shape. It is generally found that subjects will not tolerate unit doses of greater than about 1g to 2g and the young and elderly, with whom compliance is a greater problem, will generally require smaller still unit doses, e.g. 500mg. It is, however, preferable to administer the required quantity of composition in as few unit doses as possible and a balance may need to be struck between the size of the unit dose and the number of units to be administered.

The composition will generally be adapted to provide a dosage of between 0.5g and 10g of omega 3 fatty acid. In a preferred embodiment of the invention, the fatty acid is administered in a dosage of greater than about 0.1g, preferably greater than about 1g and particularly preferably greater than about 2g. Particularly preferred are dosages of less than about 10g, preferably less than about 6g, e.g. between 0.1g and 10g and particularly preferably between 1g and 4g. These dosages are calculated on the basis of the fatty acid as a free fatty acid, i.e. one preferred embodiment the above-mentioned dosages represent the dose of active fatty acid, e.g. an omega 3 fatty acid, in the administered composition.

Alternatively, if multiple fatty acid components (especially active components) are present in
the composition for administration, each component may be present in the above-mentioned amounts.

The dosage regime for the compositions of the invention may comprise the administration of the daily dosage at one time (i.e. after a morning meal) or at a plurality of occasions throughout the day (i.e. half of the daily dosage in the morning and half in the evening). If a multiple time-point administration regime is to be followed, the daily dosage is preferably divided into dosages that provide the required amount of composition at each time-point. For example, a routine of three equal dosages during the day might be conveniently administered as three unit dosages each of one third of the daily dose, or as six unit dosages each of one sixth of the daily dose, etc. In a preferred embodiment, a composition according to the invention is adapted for administration in a single daily dosage.

Preferably at least 5% by weight of the composition comprising the fatty acid (excluding the weight of a coating on a capsule, or the like) is omega 3 fatty acid, especially at least 20% and most preferably at least 40%.

Mixtures of fatty acids for administration may be encapsulated together - i.e. mixed together, or encapsulated non-mixed - wherein the dosage form would comprise one or more capsules of each substantially pure component separately.

The composition for administration may comprise one or more other active agents, e.g. vitamins such as vitamins A, D, E, mineral supplements such as iron, magnesium and zinc and other active compounds such as carotenoids, e.g. lutein and zeaxanthin.

A further aspect of the invention is accordingly directed towards nutraceutical compositions comprising the compositions described herein. According to this aspect, the invention provides a nutraceutical composition comprising a food-grade fatty-acid extract, said composition preferably further comprising one or more physiologically tolerable carriers, diluents and/or excipients. The nutraceutical composition is preferably in the form of a capsule.

The food-grade extracts are typically combined, e.g. non-chemically, with known excipients such as binders, gelling agents, lubricants, flow agents, colours, antioxidants, flavours, stabilisers etc. to form the unit dosage. These unit dosages may then be coated or otherwise finished the increase their storage stability, e.g. their resistance to oxidation.
Suitable excipients and coatings as well as methods for the preparation of nutraceutical compositions are well known in the art.

A kit is also contemplated which comprises one or more of the compositions described herein, or the nutraceutical preparations thereof, preferably adapted to provide daily dosages of the composition in as many unit dosages as make up the daily dosage. The kit comprises instructions for the administration of said composition.

Squid liver oil or an oil made from visceral material of squid, at least from some parts of the world, is a superior source for the provision of high concentrates of DHA. It has previously been described that squid and squid oils contain the omega 3 fatty acids EPA and DHA, however it has not previously been appreciated that squid is an excellent source for production of extracts with very high DHA concentration and a very low DPA concentration, e.g. by a distillation process.

Furthermore, marine animals from certain areas of the world are known to contain a variety of arsenic compounds, both water soluble and lipid soluble compounds. Water soluble compounds in visceral material will not be retained in the processes of the invention and do not represent a problem for quality of the oil. Of the lipid soluble compounds, there are those that are soluble in both the neutral and polar lipid streams. Depending on the concentration of arsenic compounds in the oil produced after the initial refining steps, further steps may be desirable to reduce the concentration of said compounds to a level acceptable for human consumption. Suitable steps for the removal of arsenic compounds from the various fractions are known, and some are described above.

Disclosed herein is a refined squid oil having a phospholipid content as hereinbefore defined, e.g. greater than about 25 % (by weight of oil) of phospholipids, and a low content, for example less than about 10, e.g. less than 7, 5, 4, 3, 2, 1, 0.5 or 0.2 mg/kg of oil, of arsenic compounds. The term "arsenic compounds" is understood to comprise any species, e.g. a molecular species, that comprises one or more bound arsenic atoms. Arsenic compounds include fatty acids containing arsenic. The proportion of arsenic (e.g. mg/kg of oil) is measured by the weight of elemental arsenic in the final composition. Preferably the refined squid oil of the invention is formulated to contain greater than about 30% of phospholipids and below about 7 mg/kg arsenic. Still more preferably, the squid oil is formulated to contain above 40% phospholipids and below 4 mg/kg arsenic.
In a related embodiment, the compositions of the invention, e.g. food grade fatty acid extracts, comprise less than about 10 mg/kg, e.g. less than 7, 5, 4, 3, 2, 1, 0.5 or 0.2 mg/kg, of arsenic compounds.

The invention also provides a refined squid oil comprising greater than about 30% (by weight of oil) phospholipids and less than about 5 mg/kg of arsenic compounds, calculated by weight of elemental arsenic.

Another environmental contaminant that may be reduced through the process of the invention is cadmium. Levels of cadmium in marine products for human consumption that are considered safe are known in the art. These include 1.0 ppm in squid, e.g. squid tubes; 0.3 ppm in sword-fish and 0.1 ppm in many other fish and marine species (all levels based on wet weight). The EU has set no defined limits for cadmium levels in marine oils, however, recommended levels by current producers of marine oils is 0.1 ppm. By tailoring the conditions of the purification steps in the process of the invention, levels of cadmium compounds can be dramatically reduced, especially the levels of non-polar cadmium-containing compounds.

Accordingly, a preferred embodiment of the invention provides a refined squid oil comprising less than about 2, e.g. less than 1, 0.5, 0.3, 0.1 or 0.05 ppm of cadmium compounds. Levels of cadmium are calculated according to the weight of elemental cadmium in the final composition.

Within the scope of the invention, the term "refined squid oil" is understood to include oils comprising a majority of oil derived from squid, i.e. a minor portion of oils from other sources may be included. The presence of non-squid oils may, for example, be due to a minor portion of non-squid material being present in the source material or non-squid oils may be added during or after processing, e.g. to affect the colour, flavour, viscosity etc. of the final oil. Examples of oils that may be added to improve the characteristics of the squid oil include oils from fish and seal visceral material, especially liver, and other oils high in omega 3, omega-6 and omega-9. Preferably the total content of non-squid oil is less than 50%, e.g. less than about 30%, preferably less than about 10% and especially preferably less than about 5% by weight of oil.
The preferred species sources of squid, e.g. to prepare squid oils of the invention, are *Sepiola atlantica, Illex argentinus, Todarodes sagittatus, Gonatus sp., Todarodes pacificus* and *Dodigius gigas*.

Figure 1 exemplifies a preferred embodiment of the process for producing a food-grade oil from squid livers.

Figure 2 exemplifies a preferred embodiment of the process for producing polar and non-polar fractions from squid livers using protease digestion of the squid livers.

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the present invention.

The following examples compare products available on the market against those prepared using the processes of the invention. Selection of the raw material and use of the process described give an oil that is not comparable to other oils in the market. As indicated by Example 4, conventional processing of squid leads to considerable breakdown of the fatty acid components in the squid liver into free fatty acids. The present invention avoids the rapid development of free fatty acids in the squid liver and produces an oil lacking the high cholesterol content and loss of phospholipids which characterise conventional oil extracts. The process according to the invention produces an oil that typically contains around 0.1% cholesterol.

**Example 1**

300 ml of commercially available squid oil with a free fatty acid content (w/w) of 0.42%, was transesterified to its ethyl ester by the addition of 60 ml ethanol containing 4 ml of
a 21% solution of sodium ethoxide in ethanol. The content of DPA and DHA in the oil was 0.52% and 18.43% respectively.

The mixture was stirred at 65°C and nitrogen gas was added to the head-space of the reaction chamber. Stirring was stopped after 30 minutes and a bottom layer comprising glycerol was separated out and drained off after 30 minutes. 300 ml of water at 50°C was added for washing with careful stirring before the layers were allow to separate and the water was drained off. The washing procedure was then repeated but this time with 3% citric acid added to the water. After draining off the water, the mixture was dried on a rotary evaporator under vacuum with moderate heating.

The ethyl ester was then degassed in a molecular distillation unit at 60°C by running the oil through at moderate speed. The oil was then distilled in the same apparatus at 104 °C at moderate to low speed, and 65.1% of the oil was collected as a distillate, with an enhanced content of fatty acids of chain length 14-18 carbons. The residue, 34.9% by weight, was redistilled at 140 C and most of the oil was collected as a distillate product. The product contained 18.5% EPA, 1.53% DPA and 41.54% DHA. (Area% values by GC analysis, mg/100mg will be slightly less). The ratio of DHA to DPA in the product was thus about 27:1.

Example 2

Several products high in DHA and available in the supplement market were collected and analyzed for content of DPA and DHA. The data are provided in Table 2 as area% of total fatty acid in methyl ester form analyzed on GC-FID:

<table>
<thead>
<tr>
<th>Product</th>
<th>EPA</th>
<th>DPA</th>
<th>DHA</th>
<th>DHA/DPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pikasol-Chewing capsules</td>
<td>34.51</td>
<td>3.53</td>
<td>23.17</td>
<td>6.56</td>
</tr>
<tr>
<td>Children's DHA –Nordic Naturals</td>
<td>9.76</td>
<td>1.26</td>
<td>10.98</td>
<td>8.71</td>
</tr>
<tr>
<td>Biomega - Macronova</td>
<td>37.89</td>
<td>4.75</td>
<td>22.26</td>
<td>4.69</td>
</tr>
<tr>
<td>Nycoplus - Nycomed</td>
<td>34.29</td>
<td>2.63</td>
<td>24.99</td>
<td>9.50</td>
</tr>
<tr>
<td>Moller</td>
<td>22.35</td>
<td>2.74</td>
<td>30.61</td>
<td>11.17</td>
</tr>
<tr>
<td>Concentrate from <em>Illex argentinus</em></td>
<td>18.46</td>
<td>1.52</td>
<td>41.54</td>
<td>27.32</td>
</tr>
</tbody>
</table>
Table 2. Fatty acid profile (Gas Chromatography analysis) in 5 consumer products from health supplement store, compared to a distillate product made from squid liver oil. (*ilex argentines*). Values are given as relative area%, mg/lOOmg will be slightly less.

As can be seen from the table, the products hitherto available in the supplement market contain significantly more DPA than products obtained from squid oil. The ratio of DHA:DPA in squid oil products is typically in the range of 20:1 to 40:1, whereas the ratios of products available on the market are typically in the area of 5:1 to 10:1.

**Example 3**

The present inventors investigated frozen squid materials to determine the optimum steps for collection of the liver and for extraction of the oil from the liver. Surprisingly the oil extracted from liver immediately after thawing of the frozen liver was very high in acid value. Table 3 shows data obtained from different species and sources of squid. The acid values do not necessarily correlate to differences in lipase activity, but they do indicate that particular processing steps may need to be included to prevent the development of high levels of free fatty acids. The observed activity of the lipases in the liver may also explain why squid liver oils available for the aquaculture market is very high in free fatty acids. In one example, the inventors obtained a freeze dried sample of liver from a squid product producer where the only lipid class found was free fatty acids. Phospholipids and triacyl glycerols were completely broken down in the samples obtained.

Table 3 below shows the acid values of oils extracted from different squid liver samples. The livers were received frozen, then thawed and extracted using the Bligh Dyer method and the acid value was measured according to a standard method by the American Oil Chemists' Society (AOCS). The acid value is approximately twice the level of the weight (percent) of free fatty acid in the sample:
Example 4

3000 grams of frozen liver from squid is freeze dried in a laboratory freeze drier. The freeze dried material is then subjected to a supercritical fluid extraction (SFE) process. Carbon dioxide is flushed through the material at a pressure of 500 bars. The quantity corresponds to 12 kg CO₂ per kilo of material. 450 grams of lipids are collected in the receiver after depressurization. Then 20% ethanol is added to the solvent, and another 160 grams (after removal of ethanol) of lipids is collected.

The neutral fraction is distilled twice in a molecular distillation plant after a degassing step to obtain a vacuum of 0.001 mbar. The free fatty acids and most of the cholesterol is removed in the distillation at 190°C. The neutral fraction is then subjected to bleaching in a batch reactor using 1% of activated bleaching clay at 60°C for 45 minutes. The remaining oil is filtered to remove the clay.

The ethanol in the polar fraction is removed in a rotary evaporator down to approximately 20% ethanol content. Then this fraction is combined with equal amount by weight of the distilled and bleached neutral fraction to yield a final product high in omega 3 and high in phospholipids. Analytical data on the product streams and final blend as well as the starting material is shown in Table 4.

By comparing final blend with the extracted lipid profile it is clearly seen that the two-stream refining process is a solution to obtaining a high value nutritional oil from squid livers. Comparison of the analytical data of the final blend with that of a lipid extract prepared using the Bligh Dyer method indicates that the process of the invention provides an extract having significantly lower levels of cholesterol and environmental contaminants.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acid value of lipids extracted from liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Todarodes sagittatus</td>
<td>48</td>
</tr>
<tr>
<td>Gonatus sp.</td>
<td>25.8</td>
</tr>
<tr>
<td>Illex argentinus (trawl catch)</td>
<td>94.9</td>
</tr>
<tr>
<td>Illex argentinus (jigg Korea)</td>
<td>46.5</td>
</tr>
<tr>
<td>Todarodes pacificus (freeze dried sample)</td>
<td>140</td>
</tr>
</tbody>
</table>

Table 3
Table 4

<table>
<thead>
<tr>
<th></th>
<th>Free fatty acids (%)</th>
<th>Cholesterol (%)</th>
<th>Arsenic (mg/kg)</th>
<th>Cadmium (ppm)</th>
<th>WHO-PCDD/F-PCB-TEQ pg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral fraction</td>
<td>11.0</td>
<td>4.0</td>
<td>20</td>
<td>0.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Polar fraction</td>
<td>0.5</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Neutral fraction after refining</td>
<td>0.5</td>
<td>1.0</td>
<td>7</td>
<td>&gt;0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Final blend</td>
<td>0.5</td>
<td>0.6</td>
<td>4</td>
<td>&gt;0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Bligh Dyer extract of lipids in liver</td>
<td>5.3</td>
<td>3.5</td>
<td>16</td>
<td>1.0</td>
<td>2.74</td>
</tr>
</tbody>
</table>


Example 5

65 grams of frozen liver from squid was cut into small pieces and filled into a flat bottom Erlenmeyer flask along with a magnetic stirrer and the flask placed in a water bath on a magnet stirrer heating plate. 3.60 grams of a 50% solution of NaOH was added for pH adjustment and then 150 microlitres of Protex 6L enzyme solution (Genencor®) was added at 60°C. The reaction was allowed to take place for 2 hours. The pH in the mixture was 9.32 and the temperature was 65°C when the reaction was stopped. pH was then adjusted to 6.16 by addition of a 50% solution of citric acid. The temperature was adjusted to above 80°C for 5 minutes to denature the added enzymes.

The material was then centrifuged at 3000x g for 5 minutes. Surprisingly, the material separated into 4 phases, listed from top:

- Red oil
- Light brown solid phase
- Black water phase with low content of solids
- Solid sediment, gray colour

The top layer of red oil could be poured off the centrifuge test tube while the next to top layer of solid plugged the tube. This layer was apparently polypeptide material, but due to high content of lipids the specific gravity was less than the water. The top layer of lipids was analysed and contained only 0.9% phospholipids. The Bligh Dyer analysis of the total starting material showed 14.4% polar lipids.

The top layer of oil represents the neutral fraction representing one product stream, while the second layer represents the second product stream (the polar lipids). This second layer may be collected and dried before the polar lipids are extracted, e.g. by using a polar solvent.
CLAIMS

1. A process for producing a food-grade fatty-acid extract from a sample of squid visceral material comprising:
   a) processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction;
   b) optionally refining one or both of the lipid fractions thus obtained; and
   c) recombining the two fractions, or portions thereof, to produce a mixture having the desired characteristics.

2. The process of claim 1 wherein step (a) comprises the use of a polar solvent, preferably ethanol, as an entrainer in a supercritical fluid extraction procedure, preferably using carbon dioxide, to provide both neutral and polar lipid fractions.

3. The process of claim 1 wherein step (a) is carried out in a two-step process comprising:
   ai) supercritical fluid extraction of the sample, preferably using carbon dioxide, to obtain a lipid extract; and
   a₂) treating the lipid extract of step (a₁) with a polar solvent, preferably ethanol, to enable the separation of an essentially neutral lipid fraction and an essentially polar lipid fraction.

4. The process of claim 1 wherein step (a) is carried out in a two-step process comprising:
   ai) subjecting the sample to enzymatic digestion by one or more proteases to obtain an essentially neutral lipid fraction and a fraction comprising peptide material and polar lipids; and
   a₄) extracting an essentially polar lipid fraction from the fraction comprising peptide material and polar lipids, optionally with or after drying of said fraction.
5. The process of any one of claims 1 to 4 further comprising the step(s) of immediate freezing and/or freeze drying of the squid visceral material before step (a), or wherein step (a) is carried out essentially immediately after the squid is caught.

6. The process of any one of claims 1 to 5 wherein the essentially polar lipid fraction comprises phospholipids.

7. The process of any preceding claim wherein step (b) comprises one or more of the following processes carried out on the essentially neutral fraction:
   bi) distillation (e.g. molecular distillation) to remove cholesterol and environmental contaminants;
   b2) deodorisation; and
   bi) treatment with adsorbents such as bleaching clay or activated charcoal.

8. The process of any one of claims 2, 3 and 5 to 7 wherein multiple separation vessels are used in collecting the effluent stream from the supercritical fluid extraction vessel.

9. The process of any preceding claim wherein step (b) comprises one or more of the following processes carried out on the polar fraction:
   b-i) concentrating the polar fraction, e.g. by evaporation under reduced pressure and/or raised temperature; and
   b2) purifying the polar fraction, preferably using flash or column chromatography.

10. The process of any preceding claim wherein a polar solvent used to separate the polar fraction in step (a) is not fully removed from the polar fraction before the recombination of step (c) and wherein the polar solvent is essentially removed following the recombination of the neutral and polar fractions.

11. The process of any preceding claim wherein in step (c) part of the neutral fraction is added to the polar fraction to produce a mixture having the desired characteristics.
12. The process of any preceding claim further comprising a step (d) of formulating the mixture into a food-grade product, e.g. a nutraceutical product, for human consumption.

13. The process of any preceding claim wherein the visceral material is or comprises squid liver.

14. A food-grade fatty-acid extract, preferably a nutraceutical extract for human consumption, from a sample of squid visceral material produced by, or producible by, a process as claimed in any one of claims 1 to 13.

15. The food-grade fatty-acid extract of claim 14 comprising greater than about 30% by weight of extract of phospholipids.

16. The food-grade fatty-acid extract of claim 14 or 15 comprising greater than about 18% by weight of extract of docosahexaenoic acid n-3 (DHA), calculated by weight of free DHA by weight of extract.

17. The food-grade fatty-acid extract of any one of claims 14 to 16 comprising less than about 1% by weight of extract of cholesterol.

18. The food-grade fatty-acid extract of any one of claims 14 to 17 comprising less than about 5 mg/kg of arsenic compounds, calculated by weight of elemental arsenic.

19. The food-grade fatty-acid extract of any one of claims 14 to 18 comprising less than about 1.5% docosapentaenoic acid n-3 (DPA).

20. The food-grade fatty-acid extract of any one of claims 14 to 19 comprising a DHA:DPA ratio of greater than about 20:1.

21. A nutraceutical composition comprising a food-grade fatty acid extract as claimed in any one of claims 14 to 20.
22. The nutraceutical composition of claim 21 further comprising one or more physiologically tolerable carriers, diluents and/or excipients.

23. The nutraceutical composition of claim 21 or 22 in capsule form.

24. A refined squid oil comprising:
   - greater than about 30% (by weight of oil) of phospholipids; and
   - less than about 5 mg/kg of arsenic compounds, calculated by weight of elemental arsenic, and/or less than about 0.1 ppm of cadmium compounds, calculated by weight of elemental cadmium.

25. A process for producing a food-grade fatty-acid extract from a sample of marine animal visceral material comprising:
   a) processing the sample to obtain an essentially neutral (non-polar) lipid fraction and an essentially polar lipid fraction;
   b) optionally refining one or both of the lipid fractions thus obtained; and
   c) recombining the two fractions, or portions thereof, to produce a mixture having the desired characteristics.
Freezing fresh cuts of squid

Drying the frozen material

Extracting the neutral lipid fraction

Oil refining process steps

Extracting the polar lipid fraction

Removing most of the solvent

Blending the fractions

Removing remaining solvent

FIG. 1
Obtaining squid liver material (fresh or thawed)

Mincing and heating to 60°C

Adjusting pH and digesting with protease(s) for 1-2 hrs

Neutral oil fraction

Refine

Polypeptide fraction without phospholipids

Polypeptide fraction with phospholipids

Extract phospholipids

FIG. 2