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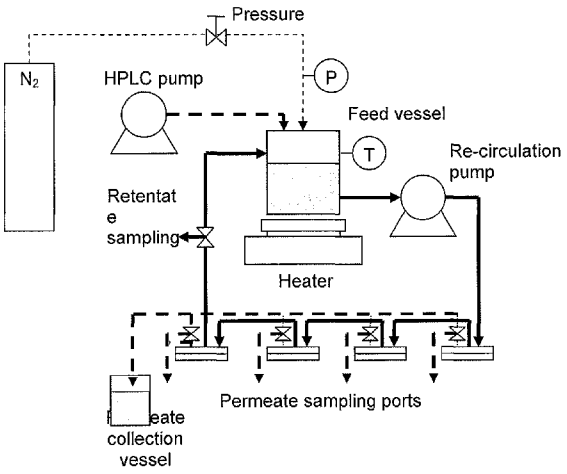

FIG. 1

(57) Abstract: The present disclosure relates generally to processes for reducing impurities and separating natural components from a marine fatty acid oil mixture using at least one selective membrane, and compositions thereof.

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MEMBRANE-BASED PROCESSES FOR REDUCING AT LEAST ONE IMPURITY AND MAKING A CONCENTRATE COMPRISING AT LEAST ONE NATURAL COMPONENT FROM A MARINE FATTY ACID OIL MIXTURE, AND COMPOSITIONS RESULTING THEREOF

[001] This application claims priority to U.S. Provisional Application No. 61/557,577, filed on November 9, 2011, which is incorporated herein by reference in its entirety.

[002] The present disclosure relates generally to a process for (1) reducing at least one impurity, i.e., undesirable natural components, such as cholesterol and aldehydes, and undesirable synthetic materials, such as dioxins, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), agrochemicals and other environmental pollutants, or (2) making a concentrate comprising at least one natural component from a marine fatty acid oil mixture such as a triglyceride or phospholipid oil using at least one selective membrane.

[003] The use of membranes to recover lipidic compounds, triacylglycerides, phospholipids, and cholesterol from egg yolk dissolved in organic solvent is discussed by C. Allègre et al. (*Cholesterol removal by nanofiltration: Applications in nutraceutics and nutritional supplements*, Journal of Membrane Science 269 (2006) 109-117). The use of membranes to degum and deacidify vegetable oil is discussed by A. Hafidi et al. (*Membrane-based simultaneous degumming and deacidification of vegetable oils*, Innovative Food Science and Emerging Technologies 6 (2005) 203-212). Moreover, U.S. Patent Application Publication No. 2010/0130761 (WO 2008/002154) describes the use of membranes for deacidifying fish oil and other glyceride oils. This disclosure utilizes the fact that free fatty acids are more easily dissolved in ethanol than triglycerides to produce an ethanol extract enriched in free fatty acids. Thereafter, a membrane is used for separation of free fatty acids from the ethanolic extract.

[004] Crude triglyceride oils commonly undergo pre-treatment processing to deliver oil having the desired content of free fatty acids, color, odor, and/or taste. Pre-treatment processing of crude triglyceride oil typically includes three process steps of deacidification, bleaching, and deodorization. Each pre-treatment processing step produces a loss of oil, and further processing steps may be necessary to remove undesirable impurities.

[005] An example of a triglyceride oil is a marine oil. Marine oils, also commonly referred to as fish oils, are a source of omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been found to regulate lipid

metabolism. Omega-3 fatty acids are useful in a number of applications, including in pharmaceutical and/or nutritional supplement products.

[006] Several formulations of omega-3 fatty acids have been developed. For example, one form of omega-3 marine fatty acid oil mixture is a concentrate of primary omega-3, long chain, polyunsaturated fatty acids from fish oil containing DHA and EPA, such as those sold under the trademark Omacor® / Lovaza™ / Zodin® / Seacor®. See, for example, U.S. Patent Nos. 5,502,077, 5,656,667, and 5,698,594. In particular, each 1000 mg capsule of Lovaza™ contains at least 90% omega-3 ethyl ester fatty acids (84% EPA/DHA); approximately 465 mg EPA ethyl ester and approximately 375 mg DHA ethyl ester.

[007] There thus remains a need in the art for a more efficient process for removing impurities from a marine fatty acid oil mixture such as a triglyceride or phospholipid oil. Disclosed herein is a process which may achieve the combined effect of one, two, or all three of the pre-treatment process steps of deacidification, bleaching, and deodorization in a single process, with the added effect of removing impurities such as, for example, cholesterol and/or environmental pollutants. The disclosed process therefore may simplify the pre-treatment of a marine fatty acid oil mixture while improving oil yield and quality. Additionally, the process may be used for making a concentrate comprising at least one natural component.

[008] The present disclosure generally relates to a process for reducing at least one impurity from a marine fatty acid oil mixture comprising: (a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution; (b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising at least one impurity; and (c) removing the organic solvent from the retentate to form a purified oil, wherein the at least one impurity in the marine oil is reduced compared to the marine fatty acid oil mixture, and the marine fatty acid oil mixture comprises marine oil chosen from triglyceride oils, phospholipid oils, and any combination thereof; and further wherein the purified oil comprises free cholesterol in an amount ranging from about 0.0 to about 2 mg/g, such as from about 0.0 to about 1 mg/g, such as from about 0.0 to about 0.5 mg/g, and/or total cholesterol in an amount less than about 2 mg/g, and wherein the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

[009] For example, the present disclosure relates to a process for reducing at least one impurity from a marine triglyceride oil comprising: (a) mixing the marine triglyceride oil with an organic solvent to form a solution, wherein the organic solvent is chosen from ethyl acetate, isopropanol, and acetone; (b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising the at least one impurity; and (c) removing the organic solvent from the retentate to form a purified marine oil, wherein the triglyceride oil comprises mono-, di- and triglycerides and the process is performed at a temperature ranging from 30 °C to 50 °C; and further wherein the purified marine oil comprises free cholesterol in an amount ranging from about 0.0 to about 0.5 mg/g, total cholesterol in an amount less than about 2 mg/g, PBDE 154 (2,2',4,4',5,6' hexabromodiphenyl ether) in amount less than about 0.02 ng/g, and comprises about 0.1 ng/g of PBDE 47 (2,2',4,4'- tetrabromodiphenyl ether); and further wherein the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}, and has a molecular weight cut-off ranging from about 200 g/mol to about 700 g/mol.

[010] In addition, the present disclosure relates to a process for reducing at least one impurity from a marine fatty acid oil mixture comprising: (a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution; (b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising at least one impurity; and (c) removing the organic solvent from the retentate to form a purified oil, wherein the at least one impurity in the marine fatty acid oil mixture is reduced compared to the purified oil, and the marine fatty acid oil mixture is chosen from triglyceride oils, phospholipid oils, and any combination thereof; and further wherein the at least one selective membrane is chosen such that it has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

[011] The present disclosure further relates to a process for making a concentrate comprising at least one natural component from a marine fatty acid oil mixture, comprising: (a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution; (b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content and a permeate forms comprising the at least one natural component; and (c) removing the organic solvent from the permeate to form a concentrate comprising the at least one natural component, wherein the at least one natural component

is chosen from fat soluble vitamins A, D, or E, cholesterol, lipophilic hormones, astaxanthin, canthaxanthin, and other carotenoids, and the marine fatty acid oil mixture is chosen from triglyceride oils, phospholipid oils, and any combination thereof; and further wherein the at least one selective membrane is chosen such that it has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{Des}.

[012] Additionally, the present disclosure relates to a purified marine oil comprising: less than about 2.0 mg/g total cholesterol, and an environmental pollutant level comprising: a maximum concentration of about 1 ng/g for the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180; a maximum concentration of about 0.1 ng/g for the sum of PBDE congener nos. 28, 47, 49, 71, 99, 100, and 154; a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g for decabromodiphenyl ether; and a maximum concentration of 1.0 pg/g for the sum of PCDD, TE 2005. Still further, the present disclosure relates to a purified oil comprising greater than about 90% triglyceride oil from marine origin comprising: less than about 2 mg/g total cholesterol; and an environmental pollutant level comprising a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g decabromodiphenyl ether.

[013] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosure, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[014] Figure 1 is a schematic of the cross-flow nanofiltration system, as described in Example 1.

[015] Figure 2 is a schematic of an embodiment of the diafiltration system disclosed herein.

[016] Figure 3 is a schematic of a variation of the diafiltration system disclosed herein.

[017] Figure 4 is a schematic of another variation of the diafiltration system disclosed herein.

DESCRIPTION

[018] Particular aspects of the disclosure are described in greater detail below. The terms and definitions as used in the present application and as clarified herein are

intended to represent the meaning within the present disclosure. The patent and scientific literature referred to herein and referenced above is hereby incorporated by reference. The terms and definitions provided herein control, if in conflict with terms and/or definitions incorporated by reference.

[019] The singular forms "a," "an," and "the" include plural reference unless the context dictates otherwise.

[020] The terms "approximately" and "about" mean to be nearly the same as a referenced number or value. As used herein, the terms "approximately" and "about" should be generally understood to encompass ± 30% of a specified amount, frequency or value.

[021] As used herein the term "acid value" of a fat or an oil means the amount of free acids presented in a fat or an oil equal to the number of milligrams of potassium hydroxide needed to neutralize one gram of the oil, i.e. that the term serves as an index of the efficiency of refining. A high acid value is thus characteristic for low quality oil or fat products.

[022] As used herein the term "cholesterol" means free cholesterol and/or esterified cholesterol. The term "total cholesterol" means the sum of free cholesterol and esterified cholesterol. Thus, for the purposes of this disclosure, a process which reduces "cholesterol" may reduce free cholesterol, esterified cholesterol, or both. A process which reduces "total cholesterol" reduces the sum of free cholesterol and esterified cholesterol.

[023] The term "fatty acid(s)" includes, e.g., short-chain and long-chain saturated and unsaturated (e.g., monounsaturated and polyunsaturated) hydrocarbons comprising one carboxylic acid group. The term "fatty acid(s)" includes, but is not limited to, omega-3 fatty acids.

[024] The term "omega-3 fatty acid(s)" includes natural and synthetic omega-3 fatty acids. An "omega-3 fatty acid oil" comprises at least one natural or synthetic omega-3 fatty acid, and may further comprise pharmaceutically-acceptable omega-3 fatty acid esters, free acids, triglycerides, derivatives, conjugates (*see, e.g.*, Zaloga et al., U.S. Publication No. 2004/0254357, and Horrobin et al., U.S. Patent No. 6,245,811, each hereby incorporated by reference), precursors, salts, and mixtures thereof. Examples of omega-3 fatty acid oils include, but are not limited to, omega-3 polyunsaturated fatty acids such as α-linolenic acid (ALA, 18:3n-3), octadecatetraenoic acid (i.e., stearidonic acid, STA, 18:4n-3), eicosatrienoic acid (ETE, 20:3n-3), eicosatetraenoic acid (ETA, 20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), heneicosapentaenoic acid (HPA, 21:5n-3), docosapentaenoic acid

(DPA, clupanodonic acid, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3); and esters of omega-3 fatty acids with glycerol such as mono-, di- and triglycerides; and esters of the omega-3 fatty acids and a primary, secondary, and/or tertiary alcohol, such as, for example, fatty acid methyl esters and fatty acid ethyl esters.

[025] The term "omega-6 fatty acid(s)" includes natural and synthetic omega-6 fatty acids. An "omega-6 fatty acid oil" comprises at least one natural or synthetic omega-6 fatty acid, and may further comprise pharmaceutically-acceptable omega-6 fatty acid esters, free acids, triglycerides, derivatives, conjugates, precursors, salts, and mixtures thereof. Examples of omega-6 fatty acid oils include, but are not limited to, omega-6 polyunsaturated, long-chain fatty acids such as linoleic acid (18:2n-6), γ-linolenic acid (18:3n-6), eicosadienoic acid (20:2n-6), dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (20:4n-6), docosadienoic acid (22:2n-6), adrenic acid (22:4n-6), and docosapentaenoic acid (i.e., osbond acid, 22:5n-6); and esters, triglycerides, derivatives, conjugates, precursors, salts, and/or mixtures thereof.

[026] The term "omega-9 fatty acid(s)" includes natural and synthetic omega-9 fatty acids. An "omega-9 fatty acid" comprises at least one natural or synthetic omega-9 fatty acid, and may further comprise pharmaceutically-acceptable omega-9 fatty acid esters, free acids, triglycerides, derivatives, conjugates, precursors, salts, and mixtures thereof. Examples of omega-9 fatty acid oils include, but are not limited to, omega-9 polyunsaturated, long-chain fatty acids such as oleic acid (18:1n−9), elaidic acid (18:1 n−9), eicosenoic acid (20:1n−9), mead acid (20:3n−9), erucic acid (22:1n−9), nervonic acid (24:1n−9) and esters, triglycerides, derivatives, conjugates, precursors, salts, and/or mixtures thereof.

[027] The analogous terms "marine fatty acid oil" and "marine based fatty acid oil" include all marine-originating oils comprising triglyceride oils, phospholipid oils, and/or mixtures thereof. The term "marine-originating" means, for example, oils obtained from species living and/or growing in a body of water, e.g., ocean - salt water and fresh/brackish water. "Marine fatty acid oils" are distinguished from the analogous terms "non-marine fatty acid oil" and "non-marine based fatty acid oil," which mean oils derived from species, for example animals or plants, not living in a body of water, e.g., ocean - salt water.

[028] The term "natural components" means natural, non-synthetic components present, for example, as an impurity in a marine fatty acid oil mixture. Some natural

components may have applications in human or animal nutrition or for user purposes. Thus, it might be of interest to isolate at least one natural component from the marine fatty acid oil mixture. In the process disclosed herein, at least one natural component may cross the membrane together with other impurities and become concentrated in the permeate. The permeate may then be removed and commercialized or further processed. The process disclosed herein thus provides an added value to the production of the target compound, i.e. the purified marine oil: it further comprises a process wherein a concentrate of at least one natural component is produced and removed as a product or an intermediate product for further processing. For the purposes of the present disclosure, the term "natural component" does not include glyceride oil, phospholipid oil, and/or fatty acids.

Marine Fatty Acid Oil Mixture

[029] A marine fatty acid oil mixture such as a triglyceride or phospholipid oil according to the present disclosure may be from marine oil(s) such as from animal oil(s) and/or non-animal oil(s) or derived thereof from any of these oils. In some embodiments of the present disclosure, the marine fatty acid oil mixture comprises at least one marine oil chosen from marine animal fat or oil, marine single cell oils, marine algae oil, marine plant-based oil, marine microbial oil, and combinations thereof. Marine oils include, for example, oil originating from fish, shellfish, krill or other crustaceans, squid, marine mammals, marine algae, marine microbes, zooplankton, and lipid compositions derived from fish. In at least one embodiment, the marine oil comprises a fish oil chosen from species of sardine/pilchard, anchovy, herring, capelin, sand eel, menhaden, tuna, mackerel, halibut, and blue whiting. In another embodiment, the fish oil comprises liver oil chosen from cod, saithe, pollock, and haddock.

[030] Marine single cell oils are often defined as oils derived from marine microbial cells and which are destined for human consumption. *See, e.g.*, Wynn and Ratledge, "Microbial oils: production, processing and markets for specialty long-chain omega-3 polyunsatutrated fatty acids," pp. 43-76 in Breivik (Ed.), *Long-Chain Omega-3 Specialty Oils*, The Oily Press, P.J. Barnes & Associates, Bridgewater UK, 2007.

[031] In further embodiments of the present disclosure, the marine fatty acid oil mixture comprises oil originating from marine bacteria or yeasts (such as, for example, from a fermentation process). Thus, in at least one embodiment, the marine fatty acid oil mixture

comprises marine oil originating from a fermentation process of marine organisms (for example, bacteria, mold, yeast, and algae).

[032] The marine oils disclosed herein are distinguished from non-marine oils, such as non-marine plant-based oils, including flaxseed oil, canola oil, mustard seed oil, and soybean oil. Non-limiting examples of non-marine oils further include vegetable oils and non-marine animal fat or oil, such as milk or butter fat, or fat-containing tissue or organs from animals such as, for instance, cattle, pig, sheep, or poultry.

[033] The marine fatty acid oil mixture comprises triglyceride oils and/or phospholipid oils, or any combination thereof. Further, the marine fatty acid oil mixture may comprise greater than 90% triglycerides or phospholipid oils. The triglyceride oils may contain free fatty acids, as well as mono- and diglycerides from hydrolysis of the triglycerides.

[034] In some embodiments, the marine fatty acid oil mixture comprises greater than 90% triglyceride or phospholipid oils. In another embodiment, the marine fatty acid oil mixture comprises at least from about 10% to about 30% by weight of omega-3 fatty acids.

[035] The marine fatty acid oil mixture may comprise various impurities in various concentrations. For example, in at least one embodiment, the marine fatty acid oil mixture may comprise any or all of the following:

- free cholesterol in an amount ranging from about 0.0 mg/g to about 10 mg/g, such as from about 0.0 mg/g to about 6 mg/g,
- total cholesterol in an amount less than about 20 mg/g, such as an amount ranging from about 0.0 mg/g to about 12 mg/g,
- PBDE 47 in a concentration ranging from about 0.1 ng/g to about 5 ng/g,
- PBDE 99 in a concentration ranging from about 0.05 ng/g to about 5 ng/g,
- PBDE 100 in a concentration ranging from about 0.05 ng/g to about 5 ng/g,
- PBDE 209 in a concentration ranging from about 0.05 ng/g to about 5 ng/g,
- a sum of concentrations of PBDE 47, PBDE 99, and PBDE 100 ranging from about 0.1 ng/g to about 10 ng/g,
- a sum of concentrations of PBDE 28, PBDE 47, PBDE 49, PBDE 71, PBDE 99, PBDE 100, and PBDE 154 ranging from about 0.2 ng/g to about 20 ng/g,
- a total PCB concentration ranging from about 5 ng/g to about 1,000 ng/g,
- a sum of concentrations of PCB 28, PCB 52, PCB 101, PCB 105, PCB 118, PCB 138, PCB 153, and PCB 180 ranging from about 2 ng/g to about 300 ng/g,

- a sum of concentrations of non-ortho PCB 77, non-ortho PCB 81, non-ortho PCB 126, and non-ortho PCB 169 ranging from about 20 pg/g to about 1700 pg/g, and/or
- a sum of dioxins (Sum PCDD, TE 2005) ranging from about 0.2 pg/g to about 20 pg/g.

[036] The above examples of impurities and impurity levels, however, are intended to be non-limiting. As discussed herein, the types and amounts of impurities in fish oils vary significantly with geography, seasons, pollution, etc. The disclosed process may be used to reduce impurities in marine fatty acid oil mixtures comprising impurities at far greater levels than those disclosed above: for instance, marine fatty acid oil mixtures comprising impurities at 20 times the levels listed above.

Membrane

[037] Suitable selective membranes for use according to the present disclosure include polymeric and ceramic membranes, and mixed polymeric/inorganic membranes. Membrane rejection, R_i, is a term of art defined as:

$$R_i = \left(1 - \frac{C_{Pi}}{C_{Ri}}\right) \text{ x } 100\% \qquad (1)$$

[038] wherein $C_{P,i}$ = concentration of species i in the permeate, "permeate" being the liquid which has passed through the membrane, and $C_{R,i}$ = concentration of species i in the retentate, "retentate" being the liquid which has not passed through the membrane. It will be appreciated that a membrane is suitable for the process disclosed herein if R(marine fatty acid oil mixture) > R(impurities). That is, the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

[039] The at least one selective membrane according to the present disclosure may be formed from any polymeric or ceramic material which provides a separating layer capable of separating the desired oil content from at least one natural impurity and/or synthetic impurity present in the marine fatty acid oil mixture. For example, the at least one selective membrane may be formed from or comprise a material chosen from polymeric materials suitable for fabricating microfiltration, ultrafiltration, nanofiltration, or reverse

osmosis membranes, including polyethylene, polypropylene, polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF), polysulfone, polyethersulfone, polyacrylonitrile, polyamide, polyimide, polyamideimide, polyetherimide, cellulose acetate, polyaniline, polypyrrole, polyetheretherketone (PEEK), polybenzimidazole, and mixtures thereof. The at least one selective membrane can be made by any technique known to the art, including sintering, stretching, track etching, template leaching, interfacial polymerization, or phase inversion. In at least one embodiment, the at least one selective membrane may be crosslinked or treated so as to improve its stability in the reaction solvents. For example, non-limiting mention may be made of the membranes described in GB2437519, the contents of which are incorporated herein by reference.

[040] In at least one embodiment, the at least one selective membrane is a composite material comprising a support and a thin, non-porous, selectively permeable layer. The thin, non-porous, selectively permeable layer may, for example, be formed from or comprise a material chosen from modified polysiloxane based elastomers including polydimethylsiloxane (PDMS) based elastomers, ethylene-propylene diene (EPDM) based elastomers, polynorbornene based elastomers, polyoctenamer based elastomers, polyurethane based elastomers, butadiene and nitrile butadiene rubber based elastomers, natural rubber, butyl rubber based elastomers, polychloroprene (Neoprene) based elastomers, epichlorohydrin elastomers, polyacrylate elastomers, polyethylene, polypropylene, polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF) based elastomers, polyetherblock amides (PEBAX), polyurethane elastomers, crosslinked polyether, polyamide, polyaniline, polypyrrole, and mixtures thereof.

[041] In another embodiment, the at least one selective membrane is prepared from an inorganic material such as, for example, silicon carbide, silicon oxide, zirconium oxide, titanium oxide, and zeolites, using any technique known to those skilled in the art such as sintering, leaching, or sol-gel processing.

[042] In a further embodiment, the at least one selective membrane comprises a polymer membrane with dispersed organic or inorganic matrices in the form of powdered solids present at amounts up to 20 wt% of the polymer membrane. Carbon molecular sieve matrices can be prepared by pyrolysis of any suitable material as described in U.S. Patent No. 6,585,802. Zeolites as described in U.S. Patent No. 6,755,900 may also be used as an inorganic matrix. Metal oxides, for example, titanium dioxide, zinc oxide, and silicon dioxide may be used, such as the materials available from Evonik Industries AG

(Germany) under their AEROSIL and ADNANO trademarks. Mixed metal oxides such as mixtures of cerium, zirconium, and magnesium oxides may also be used. In at least one embodiment, the matrices will be particles less than about 1.0 micron in diameter, for example less than about 0.1 microns in diameter, such as less than about 0.01 microns in diameter.

[043] In at least one embodiment, the at least one selective membrane comprises two membranes. In another embodiment, the at least one selective membrane comprises three membranes.

[044] In at least one embodiment, the at least one selective membrane comprises a nanofiltration membrane. As used herein, the term "nanofiltration" means membrane filtration which separates particles having molar masses ranging from about 150 to about 1,500 Da. In at least one embodiment, the pressure ranges from about 0.5 MPa to about 7 MPa. In another embodiment, the at least one selective membrane is a membrane which separates particles with molar masses ranging from about 200 to about 700 Da, such as from about 200 to about 500 Da.

[045] A non-limiting example of such a membrane is a hydrophobic membrane. For the purposes of this disclosure, a "hydrophobic" membrane is one that provides a contact angle for water of more than 70° at 25°C, as measured against the static sessile drop method. For example, in at least one embodiment, the at least one selective membrane has a contact angle for water of at least 70° at 25°C, such as a contact angle of at least 90° at 25°C.

[046] As examples of hydrophobic membranes, non-limiting mention may be made of polyimide membranes, such as those made of P84 (CAS No. 9046-51-9), P84HT (CAS No. 134119-41-8), and mixtures thereof. The polyimide membranes optionally may be crosslinked as described in GB2437519, which is incorporated herein by reference. Non-limiting mention may further be made of organic-coated polyimide membranes, such as crosslinked or non-crosslinked P84 and/or P84HT membranes, wherein the coating comprises, for example, silicone acrylates.

[047] For example, silicone acrylates which can be used to coat membranes are described in US 6,368,382, US 5,733,663, JP 62-136212, P 59-225705, DE102009047351 and EP 1741481 A1, which are incorporated by reference herein. Additionally, in one embodiment, the at least one selective membrane comprises a

combination of polyimides with silicone acrylates, e.g., as described in DE102009047351 and in EP 1741481 A1.

[048] In at least one embodiment, the at least one selective membrane has a molecular weight cut-off ranging from about 150 g/mol to about 1,500 g/mol. For the purposes of this application, molecular weight cut-off is defined according to the methodology of See-Toh et al. (2007) (Journal of Membrane Science, 291 (1-2), pp. 120-125), where the molecular weight cut-off is taken to be the molecular weight at which 90% rejection is achieved of a series of styrene oligomers. For example, in at least one embodiment, the at least one selective membrane has a molecular weight cut-off ranging from about 200 g/mol to about 700 g/mol, such as from about 300 to about 600 g/mol.

Impurities

[049] The process disclosed herein may be used to purify marine oils of impurities and/or remove impurities from marine oils to form concentrates of the impurities. Fish oil from polluted areas may contain, for example, high levels of environmental pollutants that make the oil unsuitable or "not fit" for human consumption or animal feed. The disclosed method can effectively remove a wide range of environmental pollutants, including PCB's, dioxins, and brominated flame retardants (polybrominated diphenyl ethers, or PBDEs), from such oils, thereby producing oils suitable for human consumption or use as animal and/or fish feed from highly polluted marine oils.

[050] The process disclosed herein describes separating impurities from a marine fatty acid oil mixture, resulting in oil having impurity levels within desired and/or regulatory limits for, for instance, human consumption.

[051] The concentration and composition of the impurities found in the marine fatty acid oil mixture can vary. For example, a fish oil composition may vary based on geography, species, fishing season, etc. In some instances, the impurities may be absent or below the detection limit, but if the oil is concentrated, the impurities may also be concentrated. Additionally, the methods (*e.g.*, the analytical methods) used to determine the level or concentration of the impurities found in the marine fatty acid oil mixture as well as the purified oil vary with regard to the limit of detection and limit of quantification. Although established methods, e.g., validated, may be available for some of the impurities, there may not be for others.

[052] Thus the term "impurities" includes, but is not limited to, for example, undesirable and desirable natural and unnatural components present in the crude oil, such as, for example, natural and unnatural components that are not harmful to humans and/or animals but which are not desired to be in the target product. The term "impurities" also includes natural and unnatural components unsuitable for human consumption and/or animal feed, i.e., which are harmful and/or cause a bad taste or smell. For example, some impurities have a regulatory limit for human consumption because they can bioaccumulate and thereby cause toxic, mutagenic, and/or carcinogenic effects over time. The term "impurities" also means components, such as natural components, which may be desirable to separate from a marine fatty acid oil mixture, for instance to obtain a concentrate of the components.

[053] As non-limiting examples of "impurities" for the purposes of the present disclosure, mention may thus be made of environmental pollutants, cholesterol (free and/or esterified cholesterol), oxidation products, Vitamins A, D, and E (such as alpha-, beta-, and gamma tocoherol and tocotrienols), monoglycerides, astaxanthin, canthaxanthin, other carotenoids, and components that create unwanted smell and taste in the oil, such as aldehydes and/or ketones. In at least one embodiment, the removal of components that create unwanted smell and taste result in an oil having an improved taste profile.

[054] When the marine fatty acid oil mixture is chosen from oil from krill or other crustaceans, and/or zooplankton oil, "impurities" may include astaxanthin.

[055] In at least one embodiment, the process disclosed herein produces a reduction in the level of at least one impurity present in the purified oil ranging from about 50% to about 100% compared to the marine fatty acid oil mixture, such as, for example, a reduction ranging from about 70% to about 100%, such as from about 70% to about 99%, such as from about 80% to about 100%. In another embodiment, the process disclosed herein produces a permeate comprising an increased concentration of at least one component chosen from fat soluble vitamins, cholesterol, astaxanthin, canthaxanthin, beta-carotene, and other carotenoids, relative to the marine fatty acid oil mixture. For example, in at least one such embodiment, the process produces an increased concentration of at least one component chosen from astaxanthin, Vitamin A, Vitamin D, and Vitamin E, relative to the marine fatty acid oil mixture.

[056] In at least one embodiment, the process disclosed herein produces a purified marine oil comprising free cholesterol in an amount ranging from about 0.0 mg/g to

about 2 mg/g, such as from about 0.0 mg/g to about 1 mg/g, such as from about 0.0 mg/g to about 0.5 mg/g, and/or total cholesterol in an amount less than about 2 mg/g.

[057] The term "environmental pollutants" includes, but is not limited to, for example, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethane (DDT), dioxins, furans, and nonortho-PCBs

[058] "Polycyclic aromatic hydrocarbons" or "PAHs" comprise fused aromatic rings which do not contain heteroatoms or carry substituents. As non-limiting examples of PAHs, mention may be made of acenaphthene, acenaphthylene, anthracene, benzo[a]pyrene, benz[a]anthracene, chrysene, coronene, corannulene, fluorene, fluoranthene, tetracene, naphthalene, pentacene, phenanthrene, pyrene, triphenylene, indeno(1,2,3-cd)pyrene, dibenz[az/ah]anthracene, benzo[ghi]perylene, and ovalene. *See also* Table 5 disclosed herein. According to one embodiment, the untreated oil comprises PAHs such as benzo[a]pyrene, antracene, and/or pyrene. For example, the untreated oil may comprise 0.3 ng/g benzo[a]pyrene, 0.1 ng/g antracene, and/or 2-4 ng/g pyrene.

[059] "Dioxins" refers to dioxin congeners, such as, for example, 12378-PCDD, 2378-TCDD, 123478-HCDD, 123678-HCDD, 123789-HCDD, and 1234678-HCDD. *See also* Table 5 disclosed herein. In at least one embodiment, the process disclosed herein produces a reduction of about 80% to about 99% in dioxins in the purified oil compared to the marine fatty acid oil mixture.

[060] "Furans" includes, for example, dibenzofurans, including the following congeners having 4, 5, 6, or 7 chlorine atoms: 2378-TCDF, 12378/12348-PeCDF, 23478-PeCDF, 123478/123479-HxCDF, 123678-HxCDF, 123789-HxCDF, 234678-HxCDF, 1234678-HpCDF, and 1234789-HpCDF. *See also* Table 5 disclosed herein.

[061] "Polychlorinated biphenyls" or "PCBs" includes 209 different PCB congeners, including, for instance, congener numbers 18 (2,2',5-trichlorobiphenyl), 28 (2,4,4'-trichlorobiphenyl), 31 (2,4',5-trichlorobiphenyl), 33 (2',3,4-trichlorobiphenyl), 37 (3,4,4'-trichlorobiphenyl), 47 (2,2'4,4'-tetrachlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 66 (2,3',4,4'-tetrachlorobiphenyl), 74 (2,4,4',5-tetrachlorobiphenyl), 99 (2,2'4,4',5-pentachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 105 (2,3,3',4,4'-pentachlorobiphenyl) 114 (2,3,4,4',5-pentachlorobiphenyl), 118 (2,3',4,4',5-pentachlorobiphenyl), 122 (2',3,3',4,5-pentachlorobiphenyl), 123 (2',3,4,4',5-pentachlorobiphenyl), 128 (2,2',3,3',4,4'-hexachlorobiphenyl), 138 (2,2',3,5,4',5'-

hexachlorobiphenyl), 141 (2,2',3,5,5'-hexachlorobiphenyl), 149 (2,2',3,4',5',6-hexachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl), 157 (2,3,3',4,4',5'-hexachlorobiphenyl) 167 (2,3',4,4',5,5'-hexachlorobiphenyl), 170 (2,2',3,3',4,4',5-heptachlorobiphenyl), 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl), 183 (2,2',3,4,4',5',6-heptachlorobiphenyl), 187 (2,2',3,4',5,5',6-heptachlorobiphenyl), 189 (2,3,3',4,4',5,5'-heptachlorobiphenyl), 194 (2,2',3,3',4,4',5,5'-octachlorobiphenyl), 206 (2,2',3,3',4,4',5,5',6-nonachlorobiphenyl), and 209 (decachlorobiphenyl). *See also* Tables 5, 13, and 17, disclosed herein. According to one embodiment, the marine fatty acid oil mixture comprises PCBs in concentrations of 5-20 ng/g. In at least one embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 3 ng/g of a total concentration of PCBs. For example, in at least one embodiment, the purified oil comprises a maximum concentration of about 1 ng/g of a sum of the concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180.

[062] "Nonortho-PCBs" includes, for example, 33'44'-TeCB (PCB-77), 344'5-TeCB (PCB-81), 33'44'5-PeCB (PCB-126), and 33'44'55'-HCB (PCB-169). In at least one embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 30 ng/g of a sum of the concentrations of non-ortho PCB 77, non-ortho PCB 81, non-ortho PCB 126, and non-ortho PCB 169.

[063] "Polybrominated diphenyl ethers" or "PBDEs" includes 209 different congeners, including, for instance congener numbers 28 (2,4,4'-tribromodiphenyl ether), 47 (2,2',4,4'-tetrabromodiphenyl ether), 66 (2,3',4,4'-tetrabromodiphenyl ether), 49+71 (2,2',4,5'+2,3',4',6-tetrabromodiphenyl ether), 77 (3,3',4,4'-tetrabromodiphenyl ether), 85 (2,2',3,4,4'-pentabromodiphenyl ether), 99 (2,2',4,4',5-pentabromodiphenyl ether), 100 (2,2'4,4',6-pentabromodiphenyl ether), 119 (2,3',4,4',6-penbromodiphenyl ether), 138 (2,2',3,4,4',5'-hexbromodiphenyl ether), 153 (2,2',4,4',5,5'-hexabromodiphenyl ether), 154 (2,2',4,4',5,6'-hexabromodiphenyl ether), 183 (2,2',3,4,4',5',6-heptabromodiphenyl ether), 196 (2,2',3,3',4,4',5,6'-octabromodiphenyl ether), 206 (2,2',3,3',4,4',5,5',6'-nonabromodiphenyl ether), and 209 (decabromodiphenyl ether). *See also* Table 5 disclosed herein. According to one embodiment, the marine fatty acid oil mixture comprises PBDBs in concentrations of 0.1-3 ng/g. In at least one embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 0.1 ng/g of PBDE 47. In another embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 0.05 ng/g of PBDE 99. In yet another

embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 0.05 ng/g of PBDE 100. In another embodiment, the process disclosed herein produces a purified marine oil comprising a maximum concentration of about 0.02 ng/g of PBDE 154. In a further embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 0.5 ng/g of PBDE 209. In yet another embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration about 0.1 ng/g of a sum of the concentrations of PBDE 47, PBDE 99, and PBDE 100. In at least one embodiment, the process disclosed herein produces a purified oil comprising a maximum concentration of about 0.1 ng/g of a sum of the concentrations of PBDE 28, PBDE 47, PBDE 49, PBDE 71, PBDE 99, PBDE 100, and PBDE 154.

[064] "Hexachlorocyclohexanes" or "HCHs" includes, for example, the following forms: alpha-HCH, beta-HCH, gamma-HCH, and delta-HCH.

[065] "DDT" refers to, for example, the following forms: o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT.

[066] "Chlorinated pesticides" includes, for example, lindane, endrin, dieldrin, aldrin, isodrin, heptachlor-exo-epoxide, heptachlor-endo-epoxide, trans-chlordane, cis-chlordane, oxy-chlordane, chlordane, heptachlor, endosulfan-1, and mirex.

[067] According to one embodiment, "environmental pollutants" includes, for example, DDT, and/or chlorinated pesticides such as lindane, and endrin. For instance, the marine fatty acid oil mixture may comprise 10-100 ng/g DDT (total), 0.1-1 ng/g lindane, and/or 3 ng/g endrin.

Process for Reducing At Least One Impurity and Process for Making a Concentrate Comprising At Lease One Natural Component

[068] Some embodiments of the present disclosure relate to a process for reducing impurities from a marine fatty acid oil mixture such as a triglyceride or phospholipid oil using at least one selective membrane. Additionally, some embodiments of the present disclosure relate to a process for making a concentrate comprising at least one natural component from a marine fatty acid oil mixture such as a triglyceride or phospholipid oil using at least one selective membrane.

[069] According to one embodiment, the marine fatty acid oil mixture is mixed with an organic solvent to form a solution of oil and solvent. The mixing may be

achieved by any technique known to one skilled in the art, such as, for example, via static inline mixer, dynamic inline mixer, and/or mixing vessel containing a mechanical stirrer. In at least one embodiment, the solvent is miscible with the marine fatty acid oil mixture and forms a solution, such as a homogeneous solution. For example, the solution may contain the oil in an amount ranging from 1 to 60% v/v, such as from 5 to 50% v/v.

[070] The term "organic solvent" includes, for example, an organic liquid with molecular weight less than 300 Daltons. The term "solvent" includes a mixture of organic solvents, as well as a mixture of organic solvents and water.

[071] By way of non-limiting example, solvents include aromatics, alkanes, ketones, glycols, chlorinated solvents, esters, ethers, amines, nitriles, aldehydes, alcohols, phenols, amides, carboxylic acids, alcohols, furans, and dipolar aprotic solvents, and mixtures thereof and with water.

[072] By way of non-limiting example, solvents include toluene, xylene, benzene, styrene, anisole, chlorobenzene, dichlorobenzene, chloroform, dichloromethane, dichloroethane, methyl acetate, ethyl acetate, butyl acetate, methyl ether ketone (MEK), methyl iso butyl ketone (MIBK), acetone, ethylene glycols, ethanol, methanol, propanol, butanol, hexane, cyclohexane, heptane, dimethoxyethane, methyl tert butyl ether (MTBE), diethyl ether, adiponitrile, N,N dimethylformamide, dimethylsulfoxide, N,N dimethylacetamide, dioxane, nitromethane, nitrobenzene, pyridine, carbon disulfide, tetrahydrofuran, methyl-tetrahydrofuran, N-methyl pyrrolidone, N-ethyl pyrrolidone, acetonitrile, and mixtures thereof and with water.

[073] For example, in some embodiments, the solvent can be chosen from aliphatic hydrocarbons, aromatic hydrocarbons, ketones, esters, and alcohols. For instance, the solvent can be chosen from pentane, hexane, heptane, toluene, acetone, methyl ethyl ketone, methyl acetate, ethyl acetate, propyl acetate, butyl acetate, isopropanol, butanol, and pentanol. In at least one embodiment, the solvent is selected from hexane, acetone, ethyl acetate, and isopropanol.

[074] Separation of the undesirable natural and synthetic impurities may be achieved through passing the solvent-oil solution across at least one selective membrane that retains the desired oil content, i.e., in the form of a retentate, and allows permeation of the undesirable impurities, i.e., in the form of a permeate. A driving force, e.g. an applied pressure, is used to permeate content through the membrane. In at least one embodiment,

the applied pressure ranges from 1 to 100 bar. For example, the applied pressure may range from 5 to 70 bar, such as from 15 to 60 bar.

[075] In one aspect, the present disclosure provides a process for reducing undesirable natural components (e.g., cholesterol and oxidation products) and undesirable synthetic materials (e.g., dioxins, PCBs, PBDEs, PAHs, agrochemicals) present in a triglyceride or phospholipid oil by mixing the oil and a suitable solvent and applying membrane filtration, comprising the steps of: (i) providing a solution of the oil dissolved in a solvent; (ii) providing a selectively permeable membrane having a first surface and a second surface; (iii) separating the oil from the undesirable species in solution by transferring the undesirable species from the first surface to the second surface across the membrane through contact of the oil solution with the first surface, wherein the pressure at the first surface is greater than the pressure at the second surface, and wherein the membrane is a selectively permeable membrane such that the membrane rejection (R_{TG}) of the oil species is greater than the rejection (R_{Imp}) of the undesirable species.

[076] The disclosed method can also be used to make a concentrate comprising at least one natural component, such as fat soluble vitamins (such as vitamins A, D, and E), cholesterol, astaxanthin, canthaxanthin, beta-carotene, and/or other carotenoids, from a marine fatty acid oil mixture using the disclosed selective membranes, resulting in the formation of a concentrate comprising the at least one natural component. The marine fatty acid oil mixture in making at least one natural component, *e.g.*, may comprise an oil originating from marine bacteria or yeasts (such as from a fermentation process).

[077] If the membrane rejection of the desired component to be concentrated (R_{Des}) is less than the membrane rejection of the triglyceride or phospholipid oil (i.e., the membrane retains the oil content while allowing the desired natural component to pass through), then the permeate will become enriched with the desired component, i.e., forming a concentrate of the at least one natural component.

[078] In at least one embodiment, the marine oil solution is contacted with the first surface of the membrane by flowing the solution tangentially across the first surface. This is commonly known as “cross flow” filtration or “tangential flow” filtration. As a result, the marine oil content is retained as the retentate, and impurities permeate through the at least one selective membrane to form permeate material. In one embodiment, the marine fatty acid oil solution is contacted with at least one surface of at least one selective membrane, for instance, two or three selective membranes. By way of non-limiting

example, the marine fatty acid oil solution may first be contacted with one surface of the first selective membrane to remove impurities that permeate through this first membrane, then the retentate comprising the marine fatty acid oil content from the first selective membrane is contacted with a first surface of a second selective membrane to remove impurities that permeate through this second membrane. The selected first and second membranes may be the same, or the selected membranes may be different in order to effect permeation of different impurities with the different membranes. It will be understood by one skilled in the art that contacting the marine fatty acid oil solution with three or more selective membranes may be necessary to provide the desired product.

[079] In a further embodiment, the marine fatty acid oil solution may be contacted with a first surface of a first selective membrane to generate a retentate comprising the marine fatty acid oil content and a permeate depleted in fatty acid oil. The permeate may contain sufficient concentration of the marine fatty acid oil that the permeate solution from the first selective membrane is then contacted with the first surface of a second selective membrane to generate a further retentate comprising the marine fatty acid oil content and a permeate stream containing the impurities. It will be clear to one skilled in the art that by processing the first permeate solution with a second membrane, the yield of the desirable marine fatty acid oil solution will be increased. Furthermore, it will be clear to one skilled in the art that process configurations including both a series of selective membranes processing the marine fatty acid oil solution and retentate comprising the marine fatty acid oil content and a series of selective membranes processing the permeate solution from any other selective membranes are feasible.

[080] Thus, in at least one embodiment, the process disclosed herein further comprises (d) mixing the retentate with an organic solvent to form a retentate solution; (e) passing the retentate solution across the at least one selective membrane, wherein a second retentate forms comprising oil content, and a second permeate forms comprising at least one impurity; and (f) removing the organic solvent from the second retentate to form a second purified oil. In yet another embodiment, the process disclosed herein further comprises (d) mixing the permeate with an organic solvent to form a permeate solution; and (e) passing the permeate solution across the at least one selective membrane, wherein a second retentate forms comprising oil content, and a second permeate forms comprising at least one impurity.

[081] In at least one embodiment, repetition of the process of mixing, passing, and removing may continue for a period of time ranging from about 10 minutes to about twenty hours. For example, in one embodiment, the repeating of the process of mixing, passing, and removing continues for a period of time ranging from about 30 minutes to about five hours. When tangential flow filtration (sometimes also referred to as "crossflow filtration") is used to pass the solution across at least one selective membrane, the process may comprise a linear velocity ranging from about 0.1 m/s to about 5 m/s, such as, for example, from about 0.5 m/s to about 3 m/s.

[082] In the process disclosed herein, diafiltration may be used to enhance the removal of impurities from the fatty acid oil solution. Diafiltration is known to those skilled in the art and is the process whereby fresh solvent is added to a solution filtered to enhance the quantity of lower molecular weight species that permeate through the membrane. Diafiltration is a liquid filtration process in which a feed liquid containing at least two solutes is in contact with a membrane and is pressurized so that some fraction of the liquid passes through the membrane, wherein at least one solute has a higher rejection on the membrane than at least one other solute. Additional liquid is fed to the pressurized side of the membrane to make up for the liquid permeating through the membrane. The ratios between the concentration of the more highly retained solute and the concentration of the less retained solute in the permeate and retentate varies dynamically, increasing in the retentate and decreasing in the permeate. Thus, in at least one embodiment, the passing of the solution across the at least one selective membrane comprises diafiltration.

[083] In at least one embodiment, the process disclosed herein comprises a combination of crossflow and diafiltration. Relative to known processes such as dead-end filtration, the combination of crossflow and diafiltration results in less fouling, less material loss, and longer lifetime of the apparatus. As a consequence, a higher efficiency can be achieved.

[084] Optionally, any remaining solvent content in the retentate is removed, resulting in the formation of a purified oil. The purified oil may then be optionally treated with at least one adsorption process comprising at least one absorbent or adsorbent to remove additional components and/or remaining impurities. For instance, in at least one embodiment, the purified oil is treated with activated carbon or another appropriate absorbent or adsorbent such as special glass, which, for example, may remove dioxins remaining in the product.

[085] For example, a diafiltration system is illustrated in Figure 2. First a batch of the marine fatty acid oil mixture solution to be processed is fed into tank 13. Pump 15 is then used to circulate the marine fatty acid oil mixture solution (14 and 16) to a membrane module housing (17) in which a module containing a suitable membrane for the separation is located. The driving force for the separation is generated by a back-pressure valve (18), which provides a filtration pressure that maintains a trans-membrane pressure difference that allows a portion of the feed fluid to transport through the membrane to generate a permeate stream (19) and a retentate stream (20). The retentate stream (20) is returned to the feed tank (13). In order to maintain a constant volume in this system, solvent is fed from reservoir 11 to feed tank 13 by pump 12 at the same rate as liquid is permeating through the membrane (19). By applying this process, impurities are flushed through the membrane whilst the oil content is retained, thus generating a purified oil.

[086] Another variation in a diafiltration is represented in Figure 3. In this schematic, V1 represents a storage vessel for the organic solvent; V2 represents a storage vessel for the marine fatty acid oil mixture feed; V3 represents a storage vessel for the solution of the processed marine fatty acid oil mixture (retentate); V4 represents a storage vessel for the solution of impurities removed from the marine fatty acid oil mixture (permeate); V5 represents a storage vessel of the processed marine fatty acid oil mixture after removal of the organic solvent (purified oil); C1 represents a thermal solvent removal technology (e.g. a flash evaporation vessel or a thin-film evaporator) to generate the solvent-free processed marine fatty acid oil mixture; F1 represents a membrane filtration unit that removes impurities from the marine fatty acid oil mixture; F2 represents a membrane filtration unit that allows recovery of the organic solvent by retaining the larger molecular weight compounds (e.g. impurities) that have permeated through the membrane in F1); and M1 represents a mixer technology (e.g. static inline mixer or mixing tank) that generates a solution of the organic solvent and the feed marine fatty acid oil mixture.

[087] Yet another representation of a diafiltration system is illustrated in Figure 4. In this schematic, V1 represents a storage vessel for the organic solvent; V2 represents a storage vessel for the marine fatty acid oil mixture feed; V3 represents a storage vessel for the solution of the processed marine fatty acid oil mixture (retentate); V4 represents a storage vessel for the solution of impurities removed from the marine fatty acid oil mixture (permeate); V5 represents a storage vessel of the processed marine fatty acid oil mixture after removal of the organic solvent (purified oil); C1 represents a thermal solvent

removal technology (e.g. a flash evaporation vessel or a thin-film evaporator) to generate the solvent-free processed marine fatty acid oil mixture; C2 represents a thermal solvent recovery technology (e.g. flash evaporation or a distillation column) that allows recovery of the organic solvent by, for example, evaporating the organic solvent relative to the lower volatility species in the solution that has permeated through the membrane in F1; F1 represents a membrane filtration unit that removes impurities from the marine fatty acid oil mixture; and M1 represents a mixer technology (e.g. static inline mixer or mixing tank) that generates a solution of the organic solvent and the feed marine fatty acid oil mixture.

[088] In at least one embodiment, solvent content in the permeate material is optionally recovered. The recovered solvent content may then be reused to dissolve the marine fatty acid oil mixture. By way of non-limiting example, the solvent may be recovered by a thermal process such as flash evaporation, distillation, or thin-film evaporation, or it may be recovered using a membrane filtration process where the impurities are retained by the filtration membrane. In addition, in at least one embodiment, the permeate material is subjected to additional processing to recover desired components. Subsequent recovery of the desired compounds may be carried out by, for example, distillation, molecular distillation, short path evaporation, or chromatographic processes, such as HPLC (high pressure liquid chromatography) or supercritical chromatography, depending on the application.

[089] Further, the crude fatty acid oil may be pre-processed in one or several steps before constituting the starting material in the membrane process as described above. An example of such a processing step is that the marine fatty acid oil mixture may be subject to washing with water and drying. The pre-processing steps of washing and drying may prevent the build-up of components in the system that can cause fouling on the membranes. As an alternative, caustic refining may be used for the same purpose.

[090] To perform the step of washing the marine fatty acid oil mixture with water and drying, for example, the marine fatty acid oil mixture may be mixed with water by a static mixer. Separation between the marine fatty acid oil mixture and water may, for instance, be performed in a centrifuge or by gravimetric separation on a tank. Residual water may then be removed, for example, under vacuum in a dryer.

[091] It is known that certain types of activated carbon can be used to remove dioxins, furans, and dioxin-like PCB's (non-ortho PCB's) from fish oil. Activated carbon,

however, may not be effective in removing other types of pollutants. Thus, activated carbon may be used in combination, for example, with steam deodorization, since steam deodorization may reduce the concentration of some of the pollutants that are not removed by activated carbon. In general steam deodorization may be effective in removing some relatively light-boiling environmental pollutants, such as DDT and many PCB's from the marine fatty acid oil mixture, while molecules with higher molecular weight, such as many PBDE's, will not be effectively removed. For all types of environmental pollutants, however, the removal rate from steam deodorization will be significantly lower than what can be achieved using the disclosed method. This may, for example, be the case for marine triglyceride oils, where deodorization temperatures must be kept lower than for deodorization of vegetable oils to reduce thermal degradation of the polyunsaturated fatty acids, even if lower temperatures will reduce the removal rate for environmental pollutants. The choice of temperature for deodorization of such oils is often a compromise between process effect and risk of formation of degradation products. Therefore, the deodorization temperature for marine oils is usually about 170° C or even higher. The process disclosed herein typically can be performed at temperatures ranging from 30 to 50°C, depending on the solubility of the marine fatty acid oil mixture in the solvent of choice (see Table 2 for examples), with excellent removal rates for pollutants. In at least one embodiment, the process may be performed at a temperature ranging from about -10 °C to about 60 °C, such as, for example, from about 25 °C to about 50 °C.

[092] The process disclosed herein can be set up to achieve good removal rates for environmental pollutants such as, for example, PCB's, PBDE's, PAH's, dioxins, furans, non-ortho-PCBs, HCH, DDT, and chlorinated pesticides combined with acceptable yields of triglyceride oils. Complete and/or near complete removal of free cholesterol can be achieved. The process can also be used to achieve significant reductions in esterified cholesterol.

[093] Thus the process disclosed herein, for example when a hydrophobic membrane of the type disclosed herein, such as with the above-specified molecular weight cut-off, is used as the at least one selective membrane, is capable of separating a broad spectrum of impurities from a marine oil. Impurities can pass through the membrane while the glyceride and phospholipid oils are retained. Relative to other known processes, these results can be achieved without the need for pretreating the oil.

[094] The disclosed method can be used to treat triglyceride oils with high acid values, for example, oils with acid values ranging from about 2 to about 25 mg KOH/g, as well as triglyceride oils with low acid values, for examples oils with acid values ranging from about 0 to about 2 mg KOH/g.

[095] Polyunsaturated fatty acids are known to be vulnerable to thermal degradation. Compared to other known methods for the removal of environmental pollutants and/or cholesterol, the method disclosed herein may be performed effectively at gentle temperature conditions. The other known methods involve higher temperatures, which may be harmful to polyunsaturated fatty acids. By way of example, membrane filtrations may be carried out at near-ambient temperature in the range -10 °C to +60 °C, which are considered to be "gentle" temperatures that minimize thermal damage on temperature-sensitive materials. Temperatures above 100 °C, and for example, temperatures above 150 °C, are considered "harmful" for omega-3 polyunsaturated fatty acids due to the rapid occurrence of oxidation and isomerization in the oil, leading to unwanted compounds that lower the quality of the oil.

[096] In addition, the method disclosed herein can be adapted to different requirements for the degree of reduction in pollutants desired. For example, more than 99% of environmental pollutants such as PCB's, DDT, and chlorinated pesticides can be removed if desired (see, for example, Tables 14 and 17, disclosed herein). By way of non-limiting example, the degree of removal of a particular impurity and/or a particular natural component may be controlled by using more or less solvent to affect a diafiltration during the impurity removal process, i.e. if more solvent is used for diafiltration, then a higher removal of impurity/natural component is achieved. As additional non-limiting examples, the removal rate of a particular impurity and/or a particular natural component may be altered by increasing or decreasing the membrane area, or by increasing or decreasing the filtration times without changing the temperature. Thus, the method disclosed herein is highly flexible: removal rates may be varied to deliver different product requirements as well as to process different starting marine fatty acid oil mixtures (which may comprise different concentrations of fatty acid oil content, environmental pollutants, and/or natural components, for example).

[097] In another embodiment, the method disclosed herein is followed by subjecting the purified triglyceride marine oil to at least one transesterification reaction with a C_1-C_4 alcohol, for instance ethanol or methanol, using a catalyst under substantially anhydrous

conditions, and thereafter subjecting the monoesters produced in the transesterification reaction to at least one distillation, for example at least one molecular- or short path distillations. This combination results in complete or near complete removal of free cholesterol by the membrane purification step, and near complete removal of esterified cholesterol in the at least one distillation step. Thereby it is possible to obtain monoesters, for instance ethyl esters, with very low concentrations of total cholesterol. Monoesters of fish oil subjected to molecular distillation without membrane purification will likely have higher concentrations of total cholesterol, because molecular distillation is less efficient at removing free cholesterol. In at least one embodiment, ethyl esters with 0-0.5 mg/g total cholesterol can be achieved. Levels of total cholesterol that can be achieved using the disclosed method will be lower than that which can be achieved using the method disclosed in, e.g., U.S. Patent Application Publication No. 2006/0134303. Monoesters of marine oils produced using the method disclosed herein can be converted to triglycerides, for instance through reactions catalyzed by commercially available enzymes (such as, for instance, Novozyme 435), producing triglycerides concentrated in omega-3 fatty acids with very low concentrations of total cholesterol, for example from about 0 mg/g to about 0.5 mg/g.

[098] Moreover, the disclosed method can effectively remove from a triglyceride or phospholipid oil about 90-100% of the free cholesterol and at the same time reduce the amount of esterified cholesterol to less than about 50% of its initial value in one process step. Other known processes (*see, e.g.,* US 7678930 B2 / WO/2004/007655) are not effective in removing esterified cholesterol from an oil in its triglyceride form. Fish oil by nature generally contains cholesterol. The level of total cholesterol in the fish oil varies depending on species, seasonal variations, fat content, etc. For example, the fat content of many pelagic fish species, such as herring, mackerel, sardine, anchovy, and capelin, etc., may vary significantly with the seasons. When fat content is high, the fish oil will contain lower concentrations of cholesterol than when the fat content is low, because the cholesterol is more diluted in the triglyceride oil when the fat content is high. Total cholesterol in fish oils typically ranges from 6 to 12 mg/g of fish oil. The distribution of the total cholesterol between free and esterified cholesterol may also vary, but in crude fish oils typically may be about 50% free cholesterol and about 50% esterified cholesterol. Natural triglyceride fish oils containing less than 2.0 mg/g esterified cholesterol are not known. Therefore, natural triglyceride oils with a concentration of not more than 2.0 mg/g total

cholesterol cannot be produced using prior art methods, unless the oil has been subjected to chemical modification steps before being reesterified back to its triglyceride form.

[099] Marine triglyceride oils generally do not contain more than about 30% of the omega-3 fatty acids EPA+DHA. To make concentrates of more than about 30% EPA+DHA, it is necessary first to split the fatty acids into separate molecules, each molecule containing only one single fatty acid. In the omega-3 industry the triglycerides of fish oil are often transformed to ethyl esters or free fatty acids before molecular distillation, urea fractionation, extraction and/or other concentration steps. In other cases, the triglycerides may be transformed to methyl esters or other forms of fatty acids. Omega-3 concentrates in the form of free fatty acids or monoesters may be used as such, or transformed back to triglycerides and/or partial glycerides, for instance by enzymatic transesterification. The disclosed method may be used for removal of environmental pollutants, cholesterol, oxidation products, etc., from all types of omega-3 concentrates, wherein the disclosed method is applied to the oil in its triglyceride form.

[0100] The disclosed method also relates to a process for reducing the amount of at least one desired natural component such as fat-soluble vitamins, and/or cholesterol in a triglyceride or phospholipid oil. The resulting concentrate(s) comprising at least one desired natural component may be used directly after removal of the solvent, or can be used as an intermediate for further purification processes, like chromatographic methods, for instance HPLC (high pressure liquid chromatography), supercritical fluid chromatography, distillation, molecular distillation, short path evaporation, thin film evaporation, extraction using a suitable solvent, a membrane process similar to the description herein, and any combination thereof.

Resulting Composition(s)

[0101] The present disclosure also relates to compositions resulting from the process disclosed herein. Such compositions may include the retentate, the purified oil, and/or the permeate material. The disclosure also relates to the purified marine oil (the retentate from the disclosed process) after transesterification with a C_1–C_4 alcohol to monoesters, followed by a type of distillation process forming concentrates of omega-3 monoesters.

[0102] For instance, in at least one embodiment, the disclosed process produces purified marine oil comprising free cholesterol in an amount ranging from about

0.0 mg/g to about 2 mg/g, such as from about 0.0 mg/g to about 1 mg/g, such as from about 0.0 mg/g to about 0.5 mg/g, and total cholesterol in an amount less than 2 mg/g. In at least one other embodiment, the disclosed process produces a 90% reduction in at least one impurity, relative to the crude oil. In yet another embodiment, the disclosed process produces a composition, such as the permeate, comprising an increased concentration of at least one of Vitamin A, Vitamin D, Vitamin E, cholesterol, astaxanthin, canthaxanthin, and other carotenoids, relative to the crude oil.

[0103] In another embodiment the disclosed process produces purified monoesters of a marine oil comprising more than about 40% EPA+DHA and comprising total cholesterol in an amount ranging from about 0 to 0.5 mg/g.

[0104] In yet another embodiment, the disclosed process produces a glyceride oil comprising a mixture of mono-, di- and triglycerides comprising more than about 40% EPA+DHA and comprising total cholesterol in an amount ranging from about 0 to about 0.5 mg/g. Such a glyceride oil may be produced, for instance, by enzymatic esterification of the monoesters.

[0105] In another embodiment, the disclosed process produces a purified marine oil comprising:

- less than 2.0 mg/g total cholesterol; and
- a lower level of at least one environmental pollutant compared to the marine fatty acid oil mixture.

[0106] For example, in at least one embodiment, the purified oil comprises a lower level of PBDE-47 (2,2',4,4'-tetrabromodiphenyl ether) compared to the marine fatty acid oil mixture.

[0107] For instance, in at least one embodiment, the disclosed process produces a natural, low concentrate, pure triglyceride marine oil comprising:

- less than about 2.0 mg/g total cholesterol (e.g., from about 0 mg/g to about 2 mg/g, such as less than about 1 mg/g total cholesterol, such as less than about 0.5 mg/g total cholesterol); and
- an environmental pollutant level comprising:
    - a maximum concentration of about 1 ng/g for the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180;
    - a maximum concentration of about 0.5 ng/g for the sum of PBDE congener nos. 28, 47, 49, 71, 99, 100, and 154;

- a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g for decabromodiphenyl ether; and
- a maximum concentration of about 1.0 pg/g for the sum of PCDD, TE 2005.

[0108] For example, in at least one embodiment, the maximum concentration of decabromodiphenyl ether ranges from about 0.1 ng/g to about 1.0 ng/g, for example, about 0.5 ng/g. In another embodiment, the maximum concentration of decabromodiphenyl ether is about 0.2 ng/g. In yet another embodiment, the decabromodiphenyl ether is PBDE-47 (2,2',4,4'- tetrabromodiphenyl ether) and its maximum concentration is about 0.1 ng/g.

[0109] In at least one embodiment, the marine oil comprises a maximum concentration of about 0.01 ng/g PBDE 100. In another embodiment, the marine oil comprises a maximum concentration of about 0.05 ng/g PBDE 99. In yet another embodiment, the marine oil comprises a maximum concentration of about 0.02 ng/g PBDE 154. In a further embodiment, the marine oil comprises a maximum concentration about 0.1 ng/g PBDE 209.

[0110] In the above composition directed to "a natural, low concentrate, pure triglyceride marine oil," the term "natural" indicates that no transesterification, short path evaporation, and/or chemical modifications to the triglyceride oil have been performed. The term "low concentrate" indicates that the sum of EPA and DHA is not more than 33 area-percent. "Area-percent" means the % of the total peaks in the GPC-chromatogram ("A%"). The term "pure triglyceride oil" indicates that the lipids in the oil comprise at least 95 A% triglycerides and partial glycerides, and/or at least 94 A% triglycerides and not more than 4 A% partial glycerides.

[0111] Furthermore, "the sum of seven PCBs" means the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180. The term "the sum of PBDEs" means the sum of PBDE congener nos. 28, 47, 49, 71, 99, 100, and 154.

[0112] In at least one embodiment, the disclosed process produces a purified oil comprising greater than about 90% triglyceride oil from marine origin, comprising:

- less than about 2.0 mg/g total cholesterol; and
- an environmental pollutant level comprising a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g decabromodiphenyl ether.

[0113] In at least one embodiment, the disclosed process produces a purified oil comprising greater than about 90% triglyceride oil from marine origin, comprising:

- less than about 0.3 mg/g free cholesterol; and

- an environmental pollutant level comprising a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g decabromodiphenyl ether.

[0114] In at least one embodiment, the disclosed process produces a purified marine oil comprising a maximum concentration of about 0.015 mg/kg for the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180.

[0115] In at least one embodiment, the disclosed process produces a purified marine oil comprising a maximum concentration of about 2 ng/g for the sum of PBDE congener nos. 28, 47, 49, 99, 100, 153, and 154.

[0116] In another embodiment, the crude oil is phospholipid and/or krill oil. In such an embodiment, the disclosed process may produce a purified oil comprising less than 2 mg/g total cholesterol and a lower level of at least one environmental pollutant compared to the crude oil. The disclosed process may also produce a composition, such as the permeate, comprising an increased concentration of astaxanthin, relative to the marine fatty acid oil mixture.

[0117] According to the process disclosed herein, the purified marine oil produced by the process may be a composition according to the European Pharmacopeia (omega-3 ethyl ester 90, omega-3 ethyl ester/triglyceride 60, fish oil monograph) criteria for specific pollutants, and comprising less than about 2 mg/g free cholesterol, such as less than about 1 mg/g free cholesterol. In a further embodiment, the process disclosed herein, optionally combined with at least one up-concentration processing step, may produce a purified oil comprising above 80 wt% omega-3 fatty acids and an at least 1:2 to 2:1 EPA:DHA ratio. Non-limiting examples of commercial fatty acid products which may be produced using the disclosed membrane process include K85EE, AGP103, OMACOR, LOVAZA, mid omega-3 concentrates from fish oil, and Pronova PURE™ products such as 10:70 EE/TG, 70:10 EE/TG, 50:30, 500:200 EE/TG, 400:200 EE/TG, 360:240 EE/TG, and 150:600 EE/TG. Moreover, high omega-3 concentrates in ethyl ester form, a combination of mono-, di-, and triglyceride form, free fatty acid form, or phospholipid form may also be produced according to the process.

EXAMPLES

Example 1: Pollutant and Impurity Removal from Crude Fish Oil

[0118] A study of different membrane performances at different pressure and using different extraction solvents was performed. The separation of impurities from crude fish oil was evaluated. To evaluate the filtration process disclosed herein, fish oil may be spiked with selected impurities prior to nanofiltration.

Materials and Methods

[0119] The METcell cross-flow filtration apparatus (Evonik Membrane Extraction Technology Ltd., London, U.K.) consisted of an 800 mL capacity feed vessel and a pumped recirculation loop through two to five cross-flow cells connected in series. The cross-flow system is shown schematically in Figure 1. The mixing in the cross-flow cells was provided by flow from the gear pump: the flow was introduced tangentially to the membrane surface at the outer diameter of the membrane disk and followed a spiral flow pattern to a discharge point at the center of the filtration cell/disk. The nanofiltration membrane disks were conditioned with the experimental solvent at the operating pressure and 30°C until a constant flux was obtained, to ensure that any preservatives/conditioning agents were washed out of the membrane, and maximum compaction of the membrane was obtained.

[0120] The test mixture was then permeated across each conditioned membrane disk at the desired operating temperature and pressure. Samples of feed permeate and retentate solutions were collected for analysis.

[0121] Baltic Sea Fish oil was provided for the study. Baltic Sea Fish oil contains high levels of environmental pollutants. In addition, it was spiked with a number of environmental pollutants to ensure concentrations which would give reliable analytical results above detection limits. Control samples of omega-3 fatty acid ethyl ester were also provided for analytical purposes.

[0122] Analysis of environmental pollutants is time-consuming and expensive. Therefore, reduction of free cholesterol was measured as a model substance during the initial screening of performance of different membrane and solvent systems.

[0123] Table 1 lists the membranes used for the study, and their respective nominal molecular weight cut-offs.

Table 1: Membranes Used.

Entry	Membrane Type	Membrane Nominal Molecular Weight Cut-Off (g/mol)	Short Name
1	DuraMem™	200	DM 200
2	DuraMem™	300	DM 300
3	DuraMem™	500	DM 500
4	PuraMem™	380	PM S 380
5	PuraMem™	280	PM 280

Analytical Methodology

Fish Oil

[0124] A sample with 8 ml of permeate and retentate was taken for dry weight purposes. For the 1:1 dilutions (oil:solvent), a sample of 4 ml of retentate and permeate was taken.

[0125] Evaporation of the solvent solution present in the fish oil was carried out in a rotovap at an evaporating temperature of 80°C, with a vacuum pump and a refrigerated recirculator, by partially submerging the rotating flask into a water bath.

[0126] The dry weight rejection of oil was calculated using the values of the dry weight mass left in the flask, i.e., the difference between the mass of the empty flask and the mass of the flask plus the fish oil.

Free Cholesterol

[0127] The free cholesterol content in a sample was analyzed by HPLC. The following equipment and materials were used:

Apparatus

Agilent 1100 Series HPLC

Column: µPorasil, 3,9 x 300 mm, part nr: WAT027477

Analytical balance (4 decimals)

HPLC Parameters

Room temperature

Mobile phase: 2.5% isopropanol in n-heptane

Flow: 1,5 ml/min.

Time: 10 min.

Injected volume: 5 µl

Wavelength: UV 205 nm

Preparation of the Mobile Phase:

A solution with 500ml of 2.5% of Isopropanol (IPA) in *n*-heptane was degassed with argon.

Free Fatty Acids

[0128] The free fatty acids content in sample was analyzed by titration, where the neutral solution of ether/alcohol (i.e., diethyl ether solvent) was replaced by methyl *tert*-butyl ether (MTBE). Ethanol was used as the alcohol for the preparation of the neutral solution.

Environmental pollutants

[0129] Environmental pollutants were analyzed by Norsk Institutt for Luftforskning, Kjeller, Norway.

Results and Discussion

[0130] Due to the solvent food grade requirements, four organic solvents were selected to verify the membrane performance. Ethanol was also tested.

Solubility test

[0131] Table 2 shows the results of the solubility test. The Baltic Sea fish oil was diluted 1:1, 1:2, 1:3, and 1:8 with organic solvents. The non-soluble solutions were heated at 30°C and then 50 °C for one hour.

Table 2: Solubility test for Baltic Sea fish oil in organic solvents.

	Ethanol					Acetone				Isopropanol				Ethyl Acetate	Hexane
Dilution	1:1	1:2	1:3	1:8	1:15	1:1	1:2	1:3	1:8	1:1	1:2	1:3	1:8	1:1	1:1
Solubility at RT	NS	NS	NS	NS	NS	S*	S*	S*	S*	NS	NS	NS	NS	S*	S*
Solubility at 30°C	NS	NS	NS	NS	NS	S	S	S	S	S*	S*	S*	S*	S	S
Solubility at 50°C	NS	NS	NS	NS	NS	S	S	S	S	S	S	S	S	S	S

RT = room temperature (≈ 20°C)
NS = non-soluble
S = soluble
S* = soluble with small un-dissolved solids

[0132] As seen in Table 2, the fish oil was not completely soluble in any solvents tested at room temperature. However, the fish oil was soluble when heated at 30°C in acetone, ethyl acetate, and hexane, though a deposit formed in the solution after 24 hours. Ethanol created a bi-phasic solution with the fish oil even when heated at 50°C. Therefore ethanol was not suitable for screening membranes.

Membrane performance

[0133] Membrane performance was evaluated by observing (i) the permeate flux through the membrane during a fixed period of time; and (ii) the rejection values of the glycerides and impurities in the permeate stream. By using these parameters, the glycerides and impurities separation efficiency was evaluated.

[0134] (i) The flux of the solvent, J (measured in L·/(m^2·hr) or LMH), was calculated using the following equation:

$$Flux,\ J = \left(\frac{V_P}{A_m\ t}\right) \qquad \text{(Equation 1)}$$

[0135] where V_p is the volume (L) permeated through the membrane; A_m is the membrane area (m^2); and t (hr) is the time taken for the volume to permeate.

[0136] (ii) Rejection of a species is used to measure the ability of the membrane to separate that species between the permeate and retentate solutions. It is defined by the following equation:

$$Rejection(\%) = \left(1 - \frac{Permeate\ concentration}{Retentate\ concentration}\right) \times 100\,\% \qquad \text{(Equation 2)}$$

Screening

[0137] For the membrane characterization, membranes were first conditioned with pure solvent at 30 bar and 30°C filtration pressure to remove the conditioning agent present in the membranes. Afterwards, any residual solvent was drained, and a fixed volume of fish oil solution and solvent was mixed and placed in the feed tank. The membranes were then tested in continuous cross-flow at 30 bar. The system was depressurized and left with the pump running overnight. The experiment was then resumed the next day using a filtration pressure of 55 bar and an operating temperature of 30°C. Permeate and retentate samples were collected after 4 hours of filtration. Retentate and permeate samples were then analyzed for each membrane to determine membrane performance. The separation performance (rejection) results achieved and the flux of the membranes during each test are described in Tables 3 and 4.

Table 3: Summary of the membrane screening analysis using different organic solvents at 30 and 55 bar.

		Acetone		Isopropanol		Hexane		Ethyl Acetate		Ethanol
	Membrane Type	30 bar	55 bar	30 bar	55 bar	30 bar	55 bar	30 bar	55 bar	30 bar
Dry Weight	DM 200	99.7†	99.8†	--	--	--	--	--	--	--
	DM 300	99.1‡	99.7†	97.1†	97.4†	--	--	90.3†	91.1‡	94.2†
	DM 500	95.2†	96.9†	74.6	77.0	54.5	45.3	73.5	67.5	T*
	PM S 380	--	--	75.8‡	74.2	85.2†	85.3†	80.0‡	82.6‡	78.0
	PM 280	--	--	--	--	50.8	62.4	--	--	--
Free Cholesterol	DM 200	99.9	99.3	--	--	--	--	--	--	--
	DM 300	99.1	98.1	98.1	98.9	--	--	75.1‡	70.7‡	82.5†
	DM 500	89.5	85.6	73.3†	81.8	21.6	35.5	38.9	12.3	T*†
	PM S 380			68.9	67.9†	55.4†	54.5†	33.4	39.2	60.4†
	PM 280	--	--	--		25.5	T*†	--	--	--
Free Fatty Acids	DM 200	96.7	95.7	--	--	--	--	--	--	--
	DM 300	83.4	99.2	74.0†	95.3	--	--	62.0‡	T*‡	82.0†
	DM 500	73.0†	95.3	59.4†	36.2†	31.7†	1.4†	41.3†	54.8†	20.1†
	PM S 380	--	--	84.2	20.1†	40.0†	78.7†	4.4‡	T*‡	23.8†
	PM 280	--	--	--	--	22.1†	28.3†	--	--	--

T* = Analysis performed, and results indicate a negative rejection value

-- = Membrane not tested

† = Membrane had good rejection for the particular analysis

‡ = Membrane and solvent selected for further investigation

Table 4. Summary of the flux results in LMH (liter/(m^2·hour).

	Acetone		Isopropanol		Hexane		Ethyl Acetate		Ethanol
Pressure	30 bar	55 bar	30 bar	55 bar	30 bar	55 bar	30 bar	55 bar	30 bar
Solvent:Fish Oil	3:1		3:1		1:1		1:1		3:1
DM 200	45.0†	77.1†	--	--	--	--	--	--	--
DM 300	70.7‡	111.4†	5.4†	13.3†	--	--	3.1‡	4.1	2.5
DM 500	61.1†	92.1†	8.6†	20.6†	12.4†	7.8†	23.1†	23.6†	4.5
PM S 380	--	--	8.8†	20.6‡	9.9†	9.0†	31.3‡	31.7‡	0.3
PM 280	--	--	--	--	1.2	0.6	--	--	--

-- = Membrane not tested

† = Membrane with good flow rate results

‡ = Membrane and solvent selected for further investigation

[0138] Regarding the retention of oil, the DM 300 and PM S 380 were found to be stable membranes, giving similar results for each solvent at different pressures.

[0139] The negative rejection results (Table 3) may be the result of an analytical error or due to a faster permeation of the compounds over the organic solvent. The PM 380 and PM 280 membranes were found to be suitable for the removal of free cholesterol and free fatty acids, due to the low rejection values.

[0140] Aside from acetone at 30 bar, the combinations of PM S 380 in isopropanol (IPA), ethyl acetate, and hexane were found to be suitable to retain the oil. DM 300 in ethyl acetate or ethanol was also an option due to the higher dry weight rejection and low rejection of impurities.

[0141] Although the membranes were tested in ethanol at 30 bar, the solution was not suitable for membrane screening due to the existence of a biphasic system. The immiscibility of IPA at room temperature and 30°C could be also a challenge during the screening process.

[0142] On the basis of rejection values and the flux of the membranes, DM 300 in acetone at 30 bar could be used, as well as PM S 380 with ethyl acetate, with isopropanol (at 55 bar), or with hexane. The flux values of hexane could be improved with a higher dilution in the feed solution.

Analytical Results

[0143] The analytical results of the screening process are provided in Tables 5 and 6. Table 5 lists the results for the retentate, while Table 6 lists the results for the permeate.

Conclusions

[0144] Acetone was found to be an efficient solvent for retaining oil. Also, DM 300 for any of the solvents presents reasonable results for dry weight reduction.

[0145] The PM S 380 was found to be a stable membrane; it showed similar rejection values for impurities and flux. This membrane was found to deliver low rejection of impurities and a rejection of >80% of oil in different solvents. Suitable combinations of membrane and solvent include the PM S 380 membrane with IPA (at 55bar), ethyl acetate, and hexane (at 30 bar).

[0146] The average theoretical molecular weight for triglycerides with the fatty acid composition of the Baltic Sea oil used in the experiments can be calculated to about 885 g/mol. The molecular weight for decabromodiphenyl ether (PBDE 209) is 959 g/mol. In spite of having a higher molecular weight than the triglycerides, analytical results showed a significant reduction of PBDEs, including PBDE 209, in the retentates (*see* Table 5; *see also* Table 9). This means that molecules with higher molecular weight than the triglyceride oil passed the membrane, while the lower molecular weight triglycerides molecules were rejected to a higher degree.

Table 5. Analytical Results for Retentate

	IUPAC No.	Unit	Fish oil before cross flow filtration	PM S 380/IPA		DM 300/Ethyl acetate		DM 500/Acetone	
				Value	% relative to incoming oil	Value	% relative to incoming oil	Value	% relative to incoming oil
Dioxins (TE, 2005)									
2378-TCDD		pg/g	0.91	0.64	70.3	0.74	81.3	0.82	90.1
12378-PECdd			1.1	0.88	80.0	0.98	89.1	1.11	100.9
123478-HxCDD		pg/g	0.02	0.02	100.0	0.01	50.0	0.02	100.0
123678-HxCDD		pg/g	0.09	0.07	77.8	0.1	111.1	0.08	88.9
123789-HxCDD		pg/g	0.02	0.02	100.0	0.01	50.0	0.02	100.0
1234678-HpCDD		pg/g	0.01	0.01	100.0	0.01	100.0	0.01	100.0
OCDD		pg/g	**0**	**0**	**n/a**	**0**	**n/a**	**0**	**n/a**
SUM PCDD		**pg/g**	**2.14**	**1.63**	**76.2**	**1.84**	**86.0**	**2.06**	**96.3**
		pg/g							
Furanes (TE, 2005)									
2378-TCDF		pg/g	0.78	0.58	74.4	0.69	88.5	0.7	89.7
12378/12348-PeCDF			0.05	0.04	80.0	0.04	80.0	0.04	80.0
23478-PeCDF		pg/g	2.8	2.21	78.9	2.51	89.6	2.56	91.4
123478/123479-HxCDF			0.08	0.06	75.0	0.07	87.5	0.09	112.5
123678-HxCDF		pg/g	0.08	0.06	75.0	0.06	75.0	0.08	100.0
123789-HxCDF		pg/g	0.01	0.01	100.0	0.01	100.0	0.01	100.0
234678-HxCDF		pg/g	0.1	0.09	90.0	0.09	90.0	0.08	80.0
1234678-HpCDF		pg/g	0	0	n/a	0	n/a	0	n/a
1234789-HpCDF		pg/g	0	0	n/a	0	n/a	0	n/a
OCDF		pg/g	0	0	n/a	0	n/a	0	n/a
SUM PCDF		pg/g	**3.91**	**3.05**	**78.0**	**3.48**	**89.0**	**3.57**	**91.3**
SUM PCDD/PCDF		**pg/g**	**6.05**	**4.68**	**77.4**	**5.32**	**87.9**	**5.63**	**93.1**
		pg/g							
nonortho-PCB (TE, 2005)									
33'3'3-TeCB (PCB-77)		pg/g	0.15	0.11	73.3	0.13	86.7	0.14	93.3
344'5-TeCB (PCB-81)			0.02	0.02	100.0	0.02	100.0	0.02	100.0
33'44'5-PeCB (PCB-126)		pg/g	11.8	9.18	77.8	1.3	87.3	10.9	92.4
33'44'55'-HCB (PCB-169)		pg/g	0.68	0.58	85.3	0.66	97.1	0.7	102.9
SUM TE-PCB		pg/g	**12.6**	**9.88**	**78.4**	**11.1**	**88.1**	**11.8**	**93.7**
		pg/g							
PCBs									

PeCB		ng/g	1.78	0.82	46.1	0.92	51.7	0.97	54.5
HCB		ng/g	17.8	9.14	51.3	10.2	57.3	11	61.8
2,2',5-TriCB	18	ng/g	27.2	14.3	52.6	16.7	61.4	18.7	68.8
2,4,4'-TriCB	28		26.2	12.5	47.7	14.5	55.3	15.7	59.9
2,4',5-TriCB	31	ng/g	28.1	14.1	50.2	16.2	57.7	18.7	66.5
2',3,4-TriCB	33	ng/g	18.1	8.72	48.2	9.99	55.2	10.7	59.1
3,4,4'-TriCB	37	ng/g	6.28	2.84	45.2	3.09	49.2	3.33	53.0
SUM TriCB		ng/g	146	72.2	49.5	83.5	57.2	92.4	63.3
		ng/g							
2,2'4,4'-TetCB	47	ng/g	13.5	6.74	49.9	7.78	57.6	7.94	58.8
2,2'5,5'-TetCB	52		39.8	19.7	49.5	23.4	58.8	25.7	64.6
2,3',4,4'-TetCB	66	ng/g	25.7	12.2	47.5	14.3	55.6	15.1	58.8
2,4,4',5-TetCB	74	ng/g	15.3	7.34	48.0	8.6	56.2	9.16	59.9
SUM TetraCB		ng/g	104	50.6	48.7	59.4	57.1	63.7	61.3
		ng/g							
2,2'4,4',5-PenCB	99	ng/g	20.2	8.64	42.8	11.1	55.0	10.8	53.5
2,2',4,5,5'-PenCB	101		50.5	20.9	41.4	25.6	50.7	26.9	53.3
2,3,3',4,4'-PenCB	105	ng/g	15.1	7.57	50.1	8.84	58.5	9.47	62.7
2,3,4,4',5-PenCB	114	ng/g	1.53	0.79	51.6	0.92	60.1	1.02	66.7
2,3',4,4',5-PenCB	118	ng/g	33.8	17.2	50.9	19.8	58.6	21.1	62.4
2',3,3',4,5-PenCB	122	ng/g	0.44	0.19	43.2	0.22	50.0	0.23	52.3
2',3,4,4',5-PenCB	123	ng/g	0.60	0.29	48.3	0.33	55.0	0.36	60.0
SUM PentaCB		ng/g	122	55.6	45.6	66.9	54.8	69.9	57.3
		ng/g							
2,2',3,3',4,4'-HexCB	128	ng/g	12.0	6.01	50.1	7.13	59.4	7.38	61.5
2,2',3,4,4',5'-HexCB	138		96.2	45	46.8	54.3	56.4	56.7	58.9
2,2',3,5,5'-HexCB	141	ng/g	14.7	7.34	49.9	8.46	57.6	8.84	60.1
2,2',3,4',5',6-HexCB	149	ng/g	52	28.6	55.0	33.4	64.2	35.7	68.7
2,2',4,4',5,5'-HexCB	153	ng/g	80.7	41	50.8	47.7	59.1	50.8	62.9
2,3,3',4,4',5-HexCB	156	ng/g	5.51	2.88	52.3	3.34	60.6	3.54	64.2
2,3,3',4,4',5'-HexCB	157	ng/g	1.00	0.5	50.0	0.62	62.0	0.63	63.0
2,3',4,4',5,5'-HexCB	167	ng/g	2.44	1.29	52.9	1.51	61.9	1.56	63.9
SUM HexaCB		ng/g	265	133	50.2	156	58.9	165	62.3
		ng/g							
2,2',3,3',4,4',5-HepCB	170	ng/g	17.2	9.03	52.5	10.5	61.0	11.5	66.9
	180		59.2	31	52.4	36.8	62.2	37.5	63.3
	183		11.3	5.49	48.6	6.34	56.1	5.8	51.3
2,2',3,4,4',5,5'-HepCB	187	ng/g	33.6	18.2	54.2	20.8	61.9	17.9	53.3
2,2',3,4,4',5',6-HepCB	189	ng/g	0.59	0.29	49.2	0.34	57.6	0.37	62.7
2,2',3,4',5,5',6-HepCB		ng/g							

2,3,3',4,4',5,5'-HepCB		ng/g	122	64	52.5	74.7	61.2	73.1	59.9
SUM HeptaCB		ng/g							
2,2',3,3',4,4',5,5'-OctCB	194	ng/g	12.8	6.94	54.2	8.26	64.5	8.51	66.5
2,2',3,3',4,4',5,5',6-NonCB	206	ng/g	5.24	3.08	58.8	3.51	67.0	3.92	74.8
DecaCB	209	ng/g	0.86	0.44	51.2	0.52	60.5	0.54	62.8
Sum 7 PCB		**ng/g**	**386**	**187**	**48.4**	**222**	**57.5**	**234**	**60.6**
Sum PCB		**ng/g**	**778**	**385**	**49.5**	**453**	**58.2**	**477**	**61.3**
PBDEs									
TBA		ng/g	4.82	2.83	58.7	2.01	41.7	3.00	62.2
2,4,4'-TriBDE	28	ng/g	0.35	0.18	51.4	0.2	57.1	0.23	65.7
2,2',4,4'-TetBDE	47	ng/g	7.86	4.06	51.7	4.6	58.5	4.90	62.3
	66		0.3	0.15	50.0	0.15	50.0	0.18	60.0
2,3',4,4'-TetBDE	49+71	ng/g	21.3	10.1	47.4	14.4	67.6	12.30	57.7
2,2',4,5'+2,3',4',6-TetBDE	77	ng/g	<0.03	0.03	n/a	0.02	n/a	0.03	n/a
3,3',4,4'-TetBDE		ng/g							
2,2',3,4,4'-PenBDE	85	ng/g	<0.05	<0.02	n/a	<0.01	n/a	<0.02	n/a
	99		1.05	0.53	56.2	0.59	56.2	0.63	60.0
2,2',4,4',5-PenBDE	100	ng/g	1.23	0.64	58.5	0.72	58.5	0.76	61.8
2,2'4,4',6-PenBDE	119	ng/g	0.05	0.06	40.0	0.02	40.0	<0.02	n/a
2,3',4,4',6-PenBDE		ng/g							
2,2',3,4,4',5'-HexBDE	138	ng/g	<0.08	<0.03	n/a	<0.01	n/a	<0.04	n/a
	153	ng/g	0.28	0.14	57.1	0.16	57.1	0.16	57.1
2,2',4,4',5,5'-HexBDE	154	ng/g	0.77	0.43	59.7	0.46	59.7	0.48	62.3
2,2',4,4',5,6'-HexBDE									
2,2',3,4,4',5',6-HepBDE	183	ng/g	0.06	0.02	33.3	0.02	33.3	<0.02	n/a
2,2',3,3',4,4',5,6'-OctBDE	196	ng/g	<0.18	<0.06	n/a	<0.02	n/a	<0.06	n/a
2,2',3,3',4,4',5,5',6'-NonBDE	206	ng/g	0.74	0.31	25.7	0.19	25.7	0.25	33.8
DecaBDE	209	ng/g	10.6	6.58	68.4	7.25	68.4	7.15	67.5
Chlorinated Pesticides									
Dieldrin		ng/g	18.9	13.5	71.4	15.4	81.5	17.8	94.2
			<1.08	<1.54	n/a	<1.77	n/a	<3.20	n/a
			<1.17	<1.54	n/a	<1.96	n/a	<3.19	n/a
Aldrin		ng/g	44.7	24.0	53.7	27.3	61.1	32.4	72.5
Isodrin		ng/g	<2.33	<2.77	n/a	<2.54	n/a	<4.21	n/a
Endrin		ng/g							
Heptachlor-exo-epoxide		ng/g							
Heptachlor-endo-epoxide		ng/g	<0.87	<0.63	n/a	<0.58	n/a	<0.96	n/a
trans-chlordane			0.95	0.68	71.6	0.79	83.2	0.85	89.5
cis-chlordane		ng/g	4.63	3.5	75.6	4.17	90.1	4.35	94.0
Oxy-chlordane			<2.52	<1.32	n/a	<2.40	n/a	<3.43	n/a

Chlordane		ng/g	<0.63	<0.67	n/a	<0.76	n/a	<0.85	n/a
Heptachlor		ng/g	<.49	<0.67	n/a	<0.81	n/a	<0.93	n/a
		ng/g							
		ng/g							
Endosulfan-I		ng/g	0.20	0.13	65.0	<0.05	<25	<0.11	<55
Mirex		ng/g	0.38						
PAHs									
Naphthalene		ng/g	24.2	19.2	79.3	17.9	74.0	18.0	74.4
Acenaphthylene		ng/g	1.36	0.85	62.5	0.81	59.6	0.90	66.2
Acenaphthene		ng/g	7.33	2.42	33.0	3.07	41.9	2.59	35.3
Fluorene		ng/g	11.4	8.28	72.6	8.90	78.1	9.35	82.0
Phenanthrene		ng/g	6.49	4.57	70.4	4.85	74.7	4.92	75.8
Anthracene		ng/g	0.84	0.62	73.8	0.67	79.8	0.63	75.0
Fluoranthene		ng/g	4.85	3.33	68.7	4.22	87.0	4.23	87.2
Pyrene		ng/g	2.03	1.72	84.7	3.90	192.1	1.83	90.1
Benz[a]anthracene		ng/g	0.47	0.42	89.7	<0.27	n/a	<0.27	n/a
Chrysene/Triphenylene		ng/g	2.30	1.42	61.7	0.98	42.6	1.03	44.8
Benzo[b/j/k]fluoranthenes		ng/g	7.06	4.26	60.3	1.91	27.1	0.99	14.0
Benzo[a]pyrene		ng/g	9.57	6.52	68.1	8.27	86.4	8.73	91.2
Indeno[1,2,3-cd]pyrene		ng/g	4.57	<0.50	<11	<0.50	<11	<0.50	<11
Dibenz[az/ah]anthracene		ng/g	6.57	<0.50	<8	<0.50	<8	<0.50	<8
Benzo[ghi]perylene		ng/g	6.16	<0.50	<8	<0.50	<8	<0.50	<8
Sum possibly carcinogenic		**ng/g**	**28.2**	**12.2**	**43.3**	**11.5**	**40.8**	**11.0**	**39.0**
Sum 16 EPA PAH		**ng/g**	**95.2**	**55.2**	**58.0**	**57.2**	**60.1**	**55.0**	**57.8**
HCH									
Alpha-HCH		ng/g	1.95	1.03	52.8	1.16	59.5	1.29	66.2
Beta-HCH			17.8	9.45	53.1	9.47	53.2	11.6	65.2
Gamma-HCH		ng/g	48.6	25.4	52.3	28.8	59.3	29.8	61.3
Delta-HCH		ng/g	<2.80	<2.42	n/a	<2.50	n/a	<1.05	n/a
		ng/g							
DDT									
o,p'-DDE		ng/g	0.98	0.53	54.1	0.73	74.5	0.67	68.4
			124	60.7	49.0	74.2	59.8	76.3	61.5
p,p'-DDE		ng/g	4.07	2.19	53.8	2.61	64.1	2.38	58.5
o,p'-DDD		ng/g	65.3	33.2	50.8	38.1	58.3	36.4	55.7
p,p'-DDD		ng/g	3.00	1.53	51.0	2.22	74.0	2.26	75.3
o,p'-DDT		ng/g	193	98.6	51.1	119	61.7	123	63.7
p,p'-DDT		ng/g	**391**	**197**	**50.4**	**237**	**60.6**	**241**	**61.6**
Sum DDT		**ng/g**							

Metals									
Hg		mg/kg	<0.005	<0.005	NP	<0.005	NP	<0.005	NP
Pb			*0.0065	0.0227		0.0107		0.0183	
Cd		mg/kg	*-0.002	*-0.002	NP	*-0.002	NP	*-0.002	NP
Cu		mg/kg	*0.0339	0.1134	NP	*0.0304	NP	0.2956	NP
Fe		mg/kg	-0.51	-0.51	NP	-0.52	NP	-0.52	NP
As		mg/kg	10.484	10.977	NP	10.299	NP	9.946	NP
Na		mg/kg	3.05	4.19	NP	2.98	NP	3.66	NP
Se		mg/kg	0.4	0.14	NP	0.11	NP	0.13	NP
*not within accredited range		mg/kg			NP		NP		NP
Other									
Free cholesterol		mg/g	6.7	9.3	138.8	4.7	70.1	5.7	85.1
Acid value		mg KOH/g	7.2	4.4	61.1	17.9	248.6	19.7	273.6

"NP" indicates that the calculation and/or measurement was not performed.
"% relative to incoming oil" was calculated as follows: 100*([Concentration in incoming solution]/[Concentration in outgoing solution])

Table 6. Analytical Results for Permeate

	Unit	Fish oil before cross flow filtration	PM S 380/IPA		DM 300/Ethyl acetate		DM 500/Acetone	
			Value	% relative to incoming oil	Value	% relative to incoming oil	Value	% relative to incoming oil
Free cholesterol	mg/g	6.7	10.9	162.7	18.1	270.1	NP	0.0
Triglycerides	A%	NP	75.6	NP	58.3	NP	NP	NP
Diglycerides	A%	NP	4.37	NP	5.67	NP	NP	NP
Monoglycerides	A%	NP	0.44	NP	1.39	NP	NP	NP
FFA (GCP)	A%	3.6	19.58	543.9	34.62	961.7	NP	NP
Acid value	mg KOH/g	7.2	NP	0.0	NP	0.0	NP	0.0

"NP" indicates that the calculation and/or measurement was not performed.
"A%" = area percentage, or the % of total peaks in the GPC chromatogram.
"% relative to incoming oil" was calculated as follows: 100*([Concentration in incoming solution]/[Concentration in outgoing solution])

Example 2: Diafiltration Process

[0147] Based on the results from the experiments in Example 1, a number of simulations were performed to assess if the identified solvent and membrane combinations would be capable of providing a viable process.

[0148] The simulations were performed using a differential mass-balance model. The process being simulated was a fed-batch constant volume diafiltration (for example, see Figure 2), which means that the model assumed that a certain volume of fish oil solution (i.e. fish oil feed plus solvent) was fed into the process at the beginning of each batch, and then a diafiltration was carried out such that the amount of fresh solvent fed into the process equaled the amount of solution permeating through the membrane (i.e. a constant volume was maintained in the process). For each simulation run, it was assumed that :

[0149] (i) 4 L of oil solution containing 1 L of fish oil and 3 L of solvent were fed to the process

[0150] (ii) the membrane area employed is constant

[0151] (iii) the membrane rejection of each component remains constant during the filtration

[0152] (iv) the permeate flux remains constant throughout the filtration

[0153] (v) the membrane rejection of a Component remains constant during the diafiltration

[0154] A list of oil components is provided in Table 7. The impurities were grouped into "Components" by taking into consideration their individual rejection values.

Table 7: List of Components Indicating the Rejection Values (%) Used in the Process Simulation for Each Membrane & Solvent Combination.

		DM 300, Ethyl Acetate (Flux = 5 $L.m^{-2}.h^{-1}$)	PM S 380, IPA (Flux = 20 $L.m^{-2}.h^{-1}$)	DuraMem 500, Acetone (Flux = 60 $L.m^{-2}.h^{-1}$)
Component A	Glycerides	95	90	99
Component B	Free fatty acids	0	0	40

Component C	Free cholesterol	0	0	0
Component D	Esterified cholesterol	0	0	0
Component E	SUM 7 PCB 2,2',4,4',5,6'-HexBDE SUM possibly carcinogenic Gamma-HCH SUM DDT	0	0	0
Component F	DecaBDE Endrin	0	0	10
Component G	SUM PCDD (Dioxins) SUM PCDF (Furanes) SUM TE-PCB Dieldrin Benzo[a]pyrene	20	0	60

[0155] The results of the simulated diafiltration process are provided in Table 8. Table 8 is structured to show the respective yields in three simulations: (1) system run until Components B-G reached 99% removal; (2) system run until Components B-F reached 95% removal; and (3) system run until Components B-F reached 90% removal.

Table 8. Diafiltration Process Results

Solvent	Membrane	NM	TA (m^2)	FV (L)	Simulation	VPP (L)	T (h)	% Removal of Component							OYR (%)	OPR (%)
								A	B	C	D	E	F	G		
Ethyl Acetate	DM 300	35	875	12000	1	81000	18.5	28.6	99.9	99.9	99.9	99.9	99.9	99.5	71.4	99.99
					2	36000	8.2	13.9	95.0	95.0	95.0	95.0	95.0	90.9	86.1	99.7
					3	27000	6.2	10.6	89.5	89.5	89.5	89.5	89.5	83.5	89.4	99.4
IPA	PM S	35	875	12000	1	81000	4.6	49.1	99.9	99.9	99.9	99.9	99.9	99.9	50.9	99.99
					2	36000	2.1	25.9	95.0	95.0	95.0	95.0	95.0	95.0	74.1	99.7
					3	27000	1.5	20.1	89.5	89.5	89.5	89.5	89.5	89.5	79.9	99.3
Acetone	DM 500	35	875	12000	1	138000	2.6	10.9	99.9	100	100	100	100	99.0	89.1	99.99
					2	93000	1.8	7.2	98.9	99.9	99.9	99.9	99.9	95.0	92.5	99.96
					3	69000	1.3	5.6	96.9	99.7	99.7	99.7	99.4	90.0	94.4	99.87

NM = Number of modules
TA = Total area
FV = Feed volume
VPP = Volume of permeate passed
T = Time of diafiltration
OYR = Oil yield in the retentate
OPR = Oil purity in the retentate

[0156] The results in Table 8 show that acetone, in combination with a DM 500 membrane, can deliver a product with better oil yield and purity in less time than ethyl acetate in combination with a DM 300 membrane, or IPA in combination with a PM S membrane.

[0157] According to the simulation (see Table 8) the system of DM 500 and acetone will for instance be able to remove 98.9 % of free fatty acids, 99.9 % of both free and total cholesterol (esterified cholesterol can be calculated by subtracting free cholesterol from total cholesterol), 95% of dioxins, furans and dioxin-like PCBs, and 99.9% of most other groups of environmental pollutants, with an oil yield of 92.5%. As seen from Table 8, the removal rates can be adjusted by adjusting the filtration times, which will also affect oil yield and throughput. These simulation results confirm that the membrane process may provide the desired reduction in impurity concentrations.

Example 3: Pollutant and Impurity Removal from Crude Fish Oil

[0158] A study was performed to test different membrane performances at different pressure, temperature and solvent:crude fish oil ratios. Ethyl acetate was used as the process solvent. The separation of impurities from crude fish oil was evaluated. To evaluate the filtration process disclosed herein, fish oil may be spiked with selected impurities prior to nanofiltration.

Materials and Methods

[0159] The METcell cross-flow filtration apparatus (Evonik Membrane Extraction Technology Ltd., London, U.K.) consisted of an 800 mL capacity feed vessel and a pumped recirculation loop through two to five cross-flow cells connected in series. The cross-flow system is shown schematically in Figure 1. The mixing in the cross-flow cells was provided by flow from the gear pump: the flow was introduced tangentially to the membrane surface at the outer diameter of the membrane disk and followed a spiral flow pattern to a discharge point at the center of the filtration cell/disk. The nanofiltration membrane disks were conditioned with the experimental solvent at the operating pressure and temperature until a constant flux was obtained, to ensure that any preservatives/conditioning agents were washed out of the membrane, and maximum compaction of the membrane was obtained.

[0160] The test mixture was then permeated across each conditioned membrane disk at the desired operating temperature and pressure. Samples of feed permeate and retentate solutions were collected for analysis.

[0161] Baltic Sea fish oil was provided for the study. Baltic Sea fish oil contains high levels of environmental pollutants. In addition, it was spiked with a number of environmental pollutants to ensure concentrations which would give reliable analytical results above detection limits. Control samples of omega-3 fatty acid ethyl ester were also provided for analytical purposes.

[0162] Analysis of environmental pollutants is often time-consuming and expensive. Therefore, reduction of free cholesterol was measured as a model substance during the initial screening of performance of different membrane and solvent systems.

[0163] Table 9 lists the membranes used for the study, and their respective nominal molecular weight cut-offs.

Table 9: Membranes Used

Membrane Type	Nominal Molecular Weight Cut-Off ($g.mol^{-1}$)	Short Name
DuraMem S XP1	500	DM S XP1
PuraMem S380	600	PM S380
DuraMem 500	500	DM 500
DuraMem 300	300	DM 300

Analytical Methodology

Fish Oil

[0164] A sample with 14 ml of permeate and retentate was taken for dry weight purposes.

[0165] Evaporation of the solvent solution present in the fish oil was carried out in a rotovap at an evaporating temperature of 65°C, with a vacuum pump and a refrigerated recirculator, by partially submerging the rotating flask into a water bath.

[0166] The dry weight rejection of oil was calculated using the values of the dry weight mass left in the flask, i.e. the difference between the mass of the empty flask and the mass of the flask plus the fish oil.

Free Cholesterol

[0167] The free cholesterol of a sample was analyzed by HPLC. The following equipment and materials were used:

Apparatus

Agilent 1100 Series HPLC

Column: µPorasil, 3,9 x 300 mm, part nr: WAT027477

Analytical balance (4 decimals)

HPLC Parameters

Room temperature

Isocratic

Normal phase

Mobile phase: 2.5% isopropanol in n-heptane

Flow: 1,0 ml/min.

Time: 10 min.

Injection volume: 5 µl

Wavelength: UV 205 nm

Preparation of the Mobile Phase:

A solution with 500ml of 2.5% of Isopropanol (IPA) in *n*-heptane was degassed with argon.

Free Fatty Acids

[0168] The free fatty acids of a sample were analyzed by titration, where the neutral solution of ether/alcohol (i.e., diethyl ether solvent) was replaced by methyl *tert*-butyl ether (MTBE). Ethanol was used for the preparation of the neutral solution.

Environmental pollutants

[0169] Environmental pollutants were analyzed by Norsk Institutt for Luftforskning, Kjeller, Norway.

Results and Discussion

Solubility test

[0170] Table 10 shows the results of the solubility test. The Baltic Sea fish oil was diluted 1:0.5 to 3:1. The non-soluble solutions were heated at 30°C and then 50°C for one hour.

Table 10: Solubility test for Baltic Sea fish oil in organic solvents.

Temperature	Ratio Solvent:Oil				
	1:0.5	1:0.3	1:1	2:1	3:1
10°C	n.s.	n.s.	n.s.	s**	--
20°C	s**	s**	s**	s	s
30°C	s*	s*	s	s	s

NS = non-soluble

S = soluble

S* = soluble with small un-dissolved solids

S** = Soluble with small un'dissolved solids after soaked at 30°C for 15 min.

[0171] As seen in Table 10, the fish oil was completely soluble at room temperature (20°C) in 2:1 and 3:1 solvent:crude fish oil ratio. The 1:1 solution was soluble at room temperature after heating for 15 min. Therefore the 1:1 ratio (or higher) solution was acceptable for the screening process.

Membrane performance

[0172] Membrane performance was evaluated by observing (i) the permeate flux through the membrane during a fixed period of time; and (ii) the rejection values of the glycerides and impurities in the permeate stream. By using these parameters, the glycerides and impurities separation efficiency was evaluated.

[0173] (i) The flux of the solvent, J (measured in $L \cdot m^{-2} \cdot hr$ or LMH), was calculated using the following equation:

$$Flux,\ J = \left(\frac{V_P}{A_m\ t} \right) \qquad \text{(Equation 1)}$$

[0174] where V_p is the volume (L) permeated through the membrane; A_m is the membrane area (m^2); and t (hr) is the time taken for the volume to permeate.

[0175] (ii) Rejection of a species is used to measure the ability of the membrane to separate that species between the permeate and retentate solutions. It is defined by the following equation:

$$Rejection(\%) = \left(1 - \frac{Permeate\ concentration}{Retentate\ concentration} \right) \times 100\,\% \qquad \text{(Equation 2)}$$

Screening

[0176] For the membrane characterization, membranes were first conditioned with pure solvent at the filtration pressure to remove the conditioning agent present in the membranes. Afterwards, any residual solvent was drained, and a fixed volume of fish oil solution and solvent was mixed and placed in the feed tank. The membranes were then tested in continuous cross-flow at the specified operating pressure and temperature. Permeate and retentate samples were collected after 4 hours of filtration. Retentate and permeate samples were then analyzed for each membrane to determine membrane performance. The separation performance (rejection) results achieved and the flux of the membranes during each test are described in Table 11.

Table 11: Summary of the membrane screening analysis using ethyl acetate as organic solvent.

	Pressure	30 bar @ 20C	30 bar @ 30C	55 bar @ 30C	30 bar @ 30C	30 bar @ 40C	30 bar @ 17C	30 bar @ 30C	55 bar @ 17C
	Solvent:Oil	1:1			2:1			3:1	
Flux [LMH]	DM 300	--	3.1	4.1	--	--	--	7.3	--
	DM 500	--	23.1	23.6	--	--	--	42.0	--
	PM S 380	--	31.3	31.7	--	--	--	35.1	--
	DM S XP1	4.4	7.8	--	17.8	20.1	15.4	19.7	33.6
Dry weight	DM 300	--	90.3	91.1	--	--	--	93.7	--
	DM 500	--	73.5	67.5	--	--	--	59.9	--
	PM S 380	--	80.1	82.6	--	--	--	80.2	--
	DM S XP1	--	85.4	--	93.0	91.3	93.3	89.6	93.6
Free Cholesterol	DM 300	--	75.1	70.7	--	--	--	90.0	--
	DM 500	--	38.9	12.3	--	--	--	16.7	--
	PM S 380	--	33.4	39.2	--	--	--	39.8	--
	DM S XP1	--	--	--	61	50.4	48	31.2	70.5
FFA	DM 300	--	62.0	0.0	--	--	--	62.4	--
	DM 500	--	41.3	54.8	--	--	--	5.2	--
	PM S 380	--	4.4	0.0	--	--	--	2.8	--
	DM S XP1	--	--	--	33.7	24.0	38.7	0.0	42.2

-- = Membrane not tested

† = Membrane had good rejection for the particular analysis

‡ = Membrane and solvent selected for further investigation

[0177] Rejection of dry weight for the DM and PM S380 membranes was too low to provide a high yield and/or the difference in rejection between cholesterol and glycerides dry weight was too small to provide efficient impurity removal. These membranes were not viable for an efficient impurity removal and recovery of a high yield of marine fatty acid oil mixture.

[0178] Using the DM S XP1 membrane, both high removal of cholesterol and good yield of glycerides was potentially achievable due to the high glycerides (dry weight) rejection and low free fatty acid rejection.

[0179] Increasing filtration pressure improved the permeate flux but reduced the separation performance. Conversely, a solution with a 1:1 ratio of fish oil to solvent would likely require a significant increase of membrane area to achieve the desired amount of produced purified fish oil.

Conclusions

[0180] Ethyl acetate/DM S XP1 at 30 bar and 17°C was viable for the Diafiltration process with a ratio of 3:1 solvent:crude fish oil. A 2:1 solution at 40°C also provided a feasible process to achieve a good yield/removal ratio using less solvent in the process.

Example 4: Diafiltration Process

[0181] A single stage diafiltration process was set-up to achieve a > 99% free cholesterol removal. To evaluate the filtration process disclosed herein, fish oil was spiked with selected impurities prior to nanofiltration.

Materials and Methods

[0182] The Bench Top Module Unit (BTMU) apparatus (Evonik Membrane Extraction Technology Ltd., London, U.K.) consisted of a 5 L capacity feed vessel and a pumped recirculation loop through a 1.8"x12" membrane module housing. The membrane module was conditioned with the process solvent at the specified pressure and temperature until a constant flux was obtained, to ensure that any preservatives/conditioning agents were washed out of the membrane, and maximum compaction of the membrane was obtained.

[0183] The test mixture, containing suitable markers, was circulated through the module housing at the desired operating temperature and pressure generating

permeate and retentate solutions. Samples of permeate and retentate solutions were collected for analysis, typically after 4h of filtration.

[0184] Baltic Sea fish oil was used in the study. Baltic Sea fish oil contains high levels of environmental pollutants. In addition, it was spiked with a number of environmental pollutants to ensure concentrations which would give reliable analytical results above detection limits. Control samples of omega-3 fatty acid ethyl ester were also provided for analytical purposes.

Analytical Methodology

Fish Oil

[0185] 14ml samples of permeate and retentate were taken for dry weight purposes. Evaporation of the solvent solution present in the fish oil was carried out in a rotovap at an evaporating temperature of 65°C, with a vacuum pump and a refrigerated recirculator, by partially submerging the rotating flask into a water bath.

[0186] The dry weight rejection of oil was calculated using the values of the dry weight mass left in the flask, i.e. the difference between the mass of the empty flask and the mass of the flask plus the fish oil.

Free Cholesterol

[0187] The free cholesterol content in a sample was analyzed by HPLC. The following equipment and materials were used:

Apparatus

Agilent 1100 Series HPLC

Column: µPorasil, 3,9 x 300 mm, part nr: WAT027477

Analytical balance (4 decimals)

HPLC Parameters

Room temperature

Mobile phase: 2.5% isopropanol in n-heptane

Flow: 1.0 ml/min.

Time: 10 min.

Injected volume: 5 µl

Wavelength: UV 205 nm

Preparation of the Mobile Phase:

A solution with 500ml of 2.5% of Isopropanol (IPA) in *n*-heptane was degassed with argon.

Environmental pollutants

[0188] Environmental pollutants were analyzed by Norsk Institutt for Luftforskning, Kjeller, Norway.

Results and Discussion

Membrane performance

[0189] Membrane performance was evaluated by observing (i) the permeate flux through the membrane during a fixed period of time; and (ii) the rejection values of the glycerides and impurities in the permeate stream. By using these parameters, the glycerides and impurities separation efficiency was evaluated.

[0190] (i) The flux of the solvent, J (measured in $L \cdot m^{-2} \cdot hr$ or LMH), was calculated using the following equation:

$$Flux,\ J = \left(\frac{V_p}{A_m\ t} \right) \qquad \text{(Equation 1)}$$

[0191] where V_p is the volume (L) permeated through the membrane; A_m is the membrane area (m^2); and t (hr) is the time taken for the volume to permeate.

[0192] (ii) Rejection of a species is used to measure the ability of the membrane to separate that species between the permeate and retentate solutions. It is defined by the following equation:

$$Rejection(\%) = \left(1 - \frac{Permeate\ concentration}{Retentate\ concentration} \right) \times 100\,\% \qquad \text{(Equation 2)}$$

Diafiltration

[0193] For the membrane characterization, the membrane module was first conditioned with pure solvent at 30 bar filtration pressure to remove the conditioning agent present in the membranes. Afterwards, any residual solvent was drained, and a 4L aliquot of 1:3 of fish oil:solvent was mixed and placed in the feed tank. The module was then operated at 30 bar and 30°C with recycle of both retentate and permeate to the feed tank until a constant flux was attained. The diafiltration methodology shown schematically in Figure 2 was then started. This process consisted of continuous permeation of the solution

rich in impurities such as free cholesterol while fresh solvent was added at the same rate as the permeate flow rate, such that the volume in the feed tank remained constant. Permeate and retentate samples were collected four times during the diafiltration. The experiment had a total of 13 Diafiltration volumes. After the 13th volume, a final sample of retentate and permeate was collected and analyzed to determine membrane performance. The separation performance achieved and the flux of the membranes during each test are described in Table 12.

Table 12: Summary of the membrane performance results during the diafiltration

	Initial	DV 4	DV 9	DV 13
Flux (LMH)	24.9	26.2	30.1	32.7
Dry Weight Rejection (%)	92.3	97.3	97.8	96.6
Free Cholesterol Rejection (%)	53.7	58.9	77.9	n/a

n/a – result not available due to an experimental error

[0194] There was an increase of dry weight rejection through all the diafiltration volumes. Also, free cholesterol rejection increased.

[0195] There was also a trend of increasing permeate flux during the purification of crude oil. Without being limited by theory, this could be due to reduced osmotic pressure on the retentate side of the membrane as the impurities were permeated through the membrane.

Analytical Results

[0196] The analytical results of the diafiltration process are provided in Tables 13, 14, and 15. Table 13 lists the results for the final product, while Tables 14 and 15 list the impurity removal results for various compounds and final yield, respectively.

Conclusions

[0197] Thirteen diafiltration volumes were enough to achieve high removal of the most critical impurities.

Table 13: Final Results for the feed solution and final product

	IUPAC no.	Unit	Fish oil before diafiltration	Retentate Value	% of incoming oil
Dioxins (TE, 2005)					
2378-TCDD		pg/g	39.7	0.19	0.5

12378-PECdd		pg/g	1.38	0.19	13.8
123478-HxCDD		pg/g	0.02	0.01	50.0
123678-HxCDD		pg/g	0.08	0.01	12.5
123789-HxCDD		pg/g	0.03	0.02	66.7
1234678-HpCDD		pg/g	0.01	0.00	0.0
OCDD		**pg/g**	**0**	**0.00**	**n/a**
SUM PCDD		**pg/g**	**41.2**	**0.42**	**1.0**
Furanes (TE, 2005)					
2378-TCDF		pg/g	0.86	0.1	11.6
12378/12348-PeCDF		pg/g	0.06	0.00	0.0
23478-PeCDF			3.15	0.03	1.0
123478/123479-HxCDF		pg/g	0.09	0.01	11.1
123678-HxCDF		pg/g	0.1	0.01	10.0
123789-HxCDF		pg/g	0.02	0.02	100.0
234678-HxCDF		pg/g	0.11	0.01	9.1
1234678-HpCDF		pg/g	0.0	0.0	n/a
1234789-HpCDF		pg/g	0.0	0.0	n/a
OCDF		pg/g	0.0	0.0	n/a
SUM PCDF		**pg/g**	**4.39**	**0.1**	**2.3**
SUM PCDD/PCDF		**pg/g**	**45.6**	**0.52**	**1.1**
nonotho-PCB (TE, 2005)					
33'3'3-TeCB (PCB-77)		pg/g	0.09	0.00	0.0
344'5-TeCB (PCB-81)		pg/g	0.01	0.00	0.0
33'44'5-PeCB (PCB-126)		pg/g	9.97	0.08	0.8
33'44'55'-HCB (PCB-169)		pg/g	0.73	0.00	0.0
SUM TE-PCB		**pg/g**	**10.8**	**0.09**	**0.8**
PCB's					
PeCB		ng/g	1.09	0.02	1.8
HCB		ng/g	11.9	0.03	0.3
2,2',5-TriCB	18	ng/g	9.72	0.02	0.2
2,4,4'-TriCB	28	ng/g	10.3	0.02	0.2
2,4',5-TriCB	31	ng/g	10.8	0.03	0.3
2',3,4-TriCB	33	ng/g	6.77	0.02	0.3
3,4,4'-TriCB	37	ng/g	2.23	<0.01	<0.4
Sum TriCB		ng/g	56.4	0.11	0.2
2,2'4,4'-TetCB	47	ng/g	5.8	0.01	0.2
2,2'''5,5'-TetCB	52	ng/g	17.7	0.03	0.2
2,3',4,4'-TetCB	66	ng/g	11.2	<0.01	<0.09
2,4,4',5-TetCB	74	ng/g	6.54	0.01	0.2
Sum TetraCB		ng/g	53.3	0.05	0.1
2,2'4,4',5-PenCB	99	ng/g	9.83	0.01	0.1
2,2',4,5,5'-PenCB	101	ng/g	24.9	0.04	0.2
2,3,3',4,4'-PenCB	105	ng/g	6.96	0.01	0.1
2,3,4,4',5-PenCB	114	ng/g	0.6	<0.01	NP
2,3',4,4',5-PenCB	118	ng/g	17.7	0.03	0.2
2',3,3',4,5-PenCB	122	ng/g	0.13	<0.01	NP
2',3,4,4',5-PenCB	123	ng/g	0.28	<0.01	NP

Sum PentaCB		ng/g	60.4	0.1	0.2
2,2',3,3',4,4'-HexCB	128	ng/g	5.25	<0.01	<0.19
2,2',3,4,4',5'-HexCB	138	ng/g	44.1	0.08	0.2
2,2',3,5,5'-HexCB	141	ng/g	6.63	<0.01	<0.15
2,2',3,4',5',6-HexCB	149	ng/g	25.1	0.05	0.2
2,2',4,4',5,5'-HexCB	153	ng/g	43.2	0.08	0.2
2,3,3',4,4',5-HexCB	156	ng/g	2.59	<0.01	<0.39
2,3,3',4,4',5'-HexCB	157	ng/g	0.45	<0.01	NP
2,3',4,4',5,5'-HexCB	167	ng/g	1.14	<0.01	NP
Sum HexaCB		ng/g	128	0.21	0.2
2,2',3,3',4,4',5-HepCB	170	ng/g	8.14	<0.01	<0.12
2,2',3,4,4',5,5'-HepCB	180	ng/g	26.2	0.06	0.2
2,2',3,4,4',5',6-HepCB	183	ng/g	4.97	0.01	0.2
2,2',3,4',5,5',6-HepCB	187	ng/g	15.1	0.03	0.2
2,3,3',4,4',5,5'-HepCB	189	ng/g	0.28	<0.01	NP
Sum HeptaCB		ng/g	54.6	0.1	0.2
2,2',3,3',4,4',5,5'-Oct-CB	194	ng/g	4.69	<0.01	<0.2
2,2,'3,3',4,4'5,5',6-NonCB	206	ng/g	2.17	<0.01	NP
DecaCB	209	ng/g	0.39	<0.01	NP
Sum 7 PCB		**ng/g**	**184**	**0.34**	**0.2**
Sum PCB		**ng/g**	**360**	**0.60**	**0.2**
PBDE's (Polybrominated Diphenyl Ethers)					
TBA		ng/g	2.59	<0.01	<0.4
2,4,4'-TriBDE	28	ng/g	0.26	<0.01	<3.8
2,2',4,4'-TetBDE	47	ng/g	12.9	0.05	0.4
2,3',4,4'-TetBDE	66	ng/g	<0.07	<0.02	NP
2,2',4,5'+2,3',4',6-TetBDE	49+71	ng/g	1.96	<0.02	<1.0
3,3',4,4'-Tet-BDE	77	ng/g	<0.05	<0.01	n/a
2,2',3,4,4'-PenBDE	85	ng/g	<0.11	<0.02	n/a
2,2',4,4',5-PenBDE	99	ng/g	0.67	0.01	1.5
2,2'4,4',6-PenBDE	100	ng/g	0.92	0.01	1.1
2,3',4,4',6-PenBDE	119	ng/g	<0.07	<0.02	NP
2,2',3,4,4',5'-HexBDE	138	ng/g	<0.17	<0.07	n/a
2,2',4,4',5,5'-HexBDE	153	ng/g	0.29	<0.05	NP
2,2',4,4',5,6'-HexBDE	154	ng/g	0.72	<0.02	<2.8
2,2',3,4,4',5',6-HepBDE	183	ng/g	<0.12	<0.05	NP
2,2',3,3',4,4',5,6'-OctBDE	196	ng/g	<0.39	<0.28	n/a
2,2',3,3',4,4',5,5',6-NonBDE	206	ng/g	0.75	<0.29	NP
DecaBDE	209	ng/g	6.30	0.61	9.7
Chlorinated Pesticides					
Dieldrin		ng/g	17	<3.42	<20
Aldrin			<0.02	<0.03	n/a
Isodrin			<0.04	<0.09	n/a
Endrin			23.8	<1.39	<5.5
Heptachlor-exo-epoxide			<1.37	<2.13	n/a
Heptachlor-endo-epoxide			<0.54	<0.75	n/a
trans-chlordane			0.87	<0.05	NP

cis-chlordane			4.19	<0.13	<3.1
Oxy-chlordane			1.39	<1.01	NP
Chlordane			<0.19	<0.44	n/a
Heptachlor			<0.08	<0.12	n/a
Endosulfan-I			0.13	<0.07	NP
Mirex			0.34	<0.04	NP
PAH's					
Naphtalene		ng/g	11.5	8.25	71.7
Acenaphtylene		ng/g	1.8	0.35	19.4
Acenaphtene		ng/g	7.13	0.46	6.5
Fluorene		ng/g	12.8	2.01	15.7
Phenantrene		ng/g	9.32	4.66	50.0
Antracene		ng/g	8.36	<0.27	NP
Fluoranthene		ng/g	6.96	0.45	6.5
Pyrene		ng/g	3.54	0.3	8.5
Benz(a)anthracene		ng/g	1.03	<0.27	NP
Chrysene/Triphenylene		ng/g	1.98	<0.27	NP
Benzo(b/j/k)fluoranthenes		ng/g	1.16	<0.27	NP
Benzo(a)pyrene		ng/g	12.3	<0.27	<2.2
Indeno(1,2,3-cd)pyrene		ng/g	<0.75	<0.50	n/a
Dibenz(az/ah)anthracene		ng/g	<0.75	<0.50	n/a
Benzo(ghi)perylene		ng/g	<0.75	<0.50	n/a
Sum possibly carcenogenic		**ng/g**	**16**	**1.81**	**11.3**
Sum 16 EPA PAH		**ng/g**	**80.1**	**19.3**	**24.1**
HCH (Hexachlorocyclohexane)					
Alpha-HCH		ng/g	1.57	<0.03	<1.9
Beta-HCH		ng/g	11.2	<0.07	<0.6
Gamma-HCH		ng/g	29.0	<0.04	<0.1
Delta-HCH		ng/g	<0.45	<0.10	n/a
DDT					
o,p'-DDE		ng/g	0.75	0.02	2.7
p,p'-DDE		ng/g	81.9	0.21	0.3
o,p'-DDD		ng/g	2.56	<0.04	<1.5
p,p'-DDD		ng/g	44.8	0.43	1.0
o,p'-DDT		ng/g	2.99	<0.08	<2.7
p,p'-DDT		ng/g	156	1.05	0.7
Sum DDT		**ng/g**	**289**	**1.84**	**0.6**
Metals					
Hg		mg/kg	<0.020*	0.14	NP
Pb		mg/kg	<0.040	<0.040	NP
Cd		mg/kg	<0.020	<0.020	NP
Cu		mg/kg	<0.10	0.29	NP
Fe		mg/kg	6.5	5.8	89.2
As		mg/kg	11	10	90.9
Na		mg/kg	<100	<100	NP
Se		mg/kg	<0.10	0.13	NP

Other parameters					
Total cholesterol		mg/g	9.31	1.29	13.9
Free cholesterol		mg/g	5.83	0	0.0
Esterified cholesterol (calculated)		mg/g	3.48	1.29	37.1
Acid value		mg KOH/g	7.4	0.29	3.9
Alpha tocoherol		mg/g	0.098	0	0.0
Vitamin A		mg/g	23.6	5.53	23.4
Vitamin D3		I.U./kg	32400	6790	21.0
TG		A%	89.7	98.1	109.4
DG		A%	3.7	1.9	51.4
MG		A%	0	0	NP
Color, Gardner			13.2	14.1	106.8
Abs. 233 nm			0.2016	0.2019	100.1
Conjugated dienes		%	0.701	0.702	100.1

"NP" indicates that the calculation and/or measurement was not performed.
"A%" = area percentage, or the % of total peaks in the GPC chromatogram.
"% relative to incoming oil" was calculated as follows: 100*([Concentration in incoming solution]/[Concentration in outgoing solution])
"*" Possibly an analytical error

Table 14: Removal of key impurities in the final product

Impurities	Unit	Feed	Product	Removal (%)
Total cholesterol	mg/g	9.31	1.29	89.4
Free cholesterol	mg/g	5.83	0	99.0
Esterified cholesterol (calculated)	mg/g	3.48	1.29	71.6
Acid value	mg KOH/g	7.4	0.29	97.0
Alpha tocoherol	mg/g	0.098	0	99.0
Vitamin A	mg/g	23.6	5.53	82.0
Vitamin D3	I.U./kg	32400	6790	83.9
SUM PCDD	pg/g	41.2	0.42	99.2
Sum 7 PCB	ng/g	184	0.34	99.9
2,2',4,4',5,6'-HexBDE	ng/g	0.72	<0.02	97.9
DecaBDE	ng/g	6.30	0.61	92.6
Sum DDT	ng/g	289	1.84	99.5
Benzo(a)pyrene	ng/g	12.3	<0.27	98.3

Table 15: Final Glycerides Yield in the Product

Compound		Feed	Product	Yield (%)
Triglycerides	A%	89.7	98.1	88.4
Diglycerides	A%	3.7	1.9	41.5
Total Glycerides	A%	93.4	100	86.6
Dry Weight yield	g/L	249.8	191.4	76.0

"A%" = area percentage, or the % of total peaks in the GPC chromatogram.

Example 5: Diafiltration Process

[0198] A multi-stage diafiltration process was evaluated for its ability to achieve a > 98% yield of glycerides with concomitant high removal of the target impurities. To evaluate the filtration process disclosed herein, fish oil was spiked with selected impurities prior to nanofiltration.

Materials and Methods

[0199] The Bench Top Module Unit (BTMU) apparatus (Evonik Membrane Extraction Technology Ltd., London, U.K.) consisted of a 5 L capacity feed vessel and a pumped recirculation loop through a 1.8" x 12" membrane module housing. The membrane module was conditioned with the experimental solvent at the operating pressure and temperature until a constant flux was obtained, to ensure that any preservatives/conditioning agents were washed out of the membrane, and maximum compaction of the membrane was obtained.

[0200] The test mixture, containing suitable marker, was circulated through the module housing at the desired operating temperature and pressure generating permeate and retentate solutions. Samples of permeate and retentate solutions were collected for analysis, typically after 4h of filtration.

[0201] Baltic Sea fish oil was provided for the study. Baltic Sea fish oil contains high levels of environmental pollutants. In addition, it was spiked with a number of environmental pollutants to ensure concentrations which would give reliable analytical results above detection limits. Control samples of omega-3 fatty acid ethyl ester were also provided for analytical purposes.

Analytical Methodology

Fish Oil

[0202] A sample with 14 ml of permeate and retentate was taken for dry weight purposes. Evaporation of the solvent solution present in the fish oil was carried out in a rotovap at an evaporating temperature of 65°C, with a vacuum pump and a refrigerated recirculator, by partially submerging the rotating flask into a water bath.

[0203] The dry weight rejection of oil was calculated using the values of the dry weight mass left in the flask, i.e. the difference between the mass of the empty flask and the mass of the flask plus the fish oil.

Free Cholesterol

[0204] The free cholesterol content in a sample was analyzed by HPLC. The following equipment and materials were used:

Apparatus

Agilent 1100 Series HPLC

Column: µPorasil, 3,9 x 300 mm, part nr: WAT027477

Analytical balance (4 decimals)

HPLC Parameters

Room temperature

Mobile phase: 2.5% isopropanol in n-heptane

Flow: 1,0 ml/min.

Time: 10 min.

Injected volume: 5 µl

Wavelength: UV 205 nm

Preparation of the Mobile Phase:

A solution with 500ml of 2.5% of Isopropanol (IPA) in *n*-heptane was degassed with argon.

Environmental pollutants

[0205] Environmental pollutants were analyzed by Norsk Institutt for Luftforskning, Kjeller, Norway.

Results and Discussion

Membrane performance

[0206] Membrane performance was evaluated by observing (i) the permeate flux through the membrane during a fixed period of time; and (ii) the rejection values of the glycerides and impurities in the permeate stream. By using these parameters, the glycerides and impurities separation efficiency was evaluated.

[0207] (i) The flux of the solvent, J (measured in $L \cdot m^{-2} \cdot hr$ or LMH), was calculated using the following equation:

$$Flux,\ J = \left(\frac{V_P}{A_m\ t} \right) \qquad \text{(Equation 1)}$$

[0208] where V_p is the volume (L) permeated through the membrane; A_m is the membrane area (m^2); and t (hr) is the time taken for the volume to permeate.

[0209] (ii) Rejection of a species is used to measure the ability of the membrane to separate that species between the permeate and retentate solutions. It is defined by the following equation:

$$Rejection(\%) = \left(1 - \frac{Permeate\,concentration}{Retentate\,concentration}\right) \times 100\,\% \qquad \text{(Equation 2)}$$

Diafiltration

[0210] For the membrane characterization, the module was first conditioned with pure solvent at 30 bar filtration pressure to remove the conditioning agent present in the membranes. Afterwards, any residual solvent was drained, and a 4L aliquot of 1:2 of fish oil:solvent was mixed and placed in the feed tank. The module was then operated at 20 bar and 40°C with recycle of both retentate and permeate to the feed tank until a constant flux was attained. The experiment was then started using the diafiltration methodology shown schematically in Figure 2. This methodology consisted of two steps. The first step was a constant volume diafiltration process using fresh solvent for washing out impurities from the feed solution. Permeate from the first filtration stage entered the second stage, the purpose of which was to recover any di- and tri-glycerides that permeated through the membrane in the first stage. The initial liters from the permeate (volume equal to the feed volume of the first stage) were considered the feed volume to the second stage, and the remaining permeate from the first stage was processed.

[0211] The experiment had a total of 12.6 Diafiltration volumes, 6.8 diafiltration volumes during the 1st stage and 5.8 diafiltration volumes during the 2nd stage. Retentate and permeate samples were collected and analyzed to determine membrane performance. The results achieved and the flux of the membranes during each test are described in Tables 16a and 16b.

Table 16a: Summary of the membrane performance results during the 1st stage of the diafiltration

	Flux (LMH)	Dry Weight Rejection (%)	Free Cholesterol Rejection (%)
Feed	13.7	90.5	44.6

DV 1	14.4	92.4	28.1
DV 2	18.0	95.2	33.6
DV 3	18.0	95.5	23.6
DV 4	18.3	95.6	9.5
DV 5	20.9	96.4	0
DV 6	20.9	96.6	0
DV 6.8	20.9	95.6	0

Table 16b: Summary of the membrane performance results during the 2nd stage of diafiltration

	Flux (LMH)	Dry Weight Rejection (%)	Free Cholesterol Rejection (%)
Feed	41.9	77.6	61.0
DV 1	36.7	84.0	57.4
DV 2	36.7	84.4	39.6
DV 3	36.7	91.9	52.4
DV 4	35.3	93.6	47.6
DV 5	36.7	94.5	36.9
DV 5.8	35.3	96.0	45.8

[0212] There was an increase of dry weight rejection through all the diafiltration volumes. However, free cholesterol rejection was stable through the diafiltration volumes. There was an approximate 15% increase in the free cholesterol rejection from the 1st stage to the 2nd stage.

[0213] There was a trend of increasing permeate flux observed during the purification of crude oil until a steady state was achieved. No significant trend in the flux was observed during the 2nd diafiltraiton stage.

Analytical Results

[0214] The analytical results of the diafiltration process are provided in Tables 17 and 18. Table 17 lists the results of the pollutants removal after the two diafiltration stages. Table 18 lists the results for other impurities and the final product yield – the experimental yield compares well with the data obtained in Examples 1-4.

Table 17: Final Results for the feed solution and final product

Pollutant	Unit	Feed	Product 1st Stage	Product 2nd Stage	Overall Removal (%)
Dioxins (TE, 2005)					

2378-TCDD	pg/g	39.7	0.47	4.89	98.1
12378-PECdd	pg/g	1.38	0.02	0.22	97.6
123478-HxCDD	pg/g	0.02	0.00	0.00	100.0
123678-HxCDD	pg/g	0.08	0.00	0.02	97.0
123789-HxCDD	pg/g	0.03	0.00	0.00	100.0
1234678-HpCDD	pg/g	0.01	0.00	0.00	100.0
OCDD	**pg/g**	**0.00**	**0.00**	**0.00**	**NP**
SUM PCDD	**pg/g**	**41.2**	**0.5**	**5.13**	**98.1**
Furanes (TE, 2005)					
2378-TCDF	pg/g	0.86	0.01	0.13	97.8
12378/12348-PeCDF	pg/g	0.06	0.00	0.01	98.0
23478-PeCDF		3.15	0.09	0.53	97.0
123478/123479-HxCDF	pg/g	0.09	0.00	0.02	97.4
123678-HxCDF	pg/g	0.10	0.00	0.02	97.6
123789-HxCDF	pg/g	0.02	0.00	0.00	100.0
234678-HxCDF	pg/g	0.11	0.00	0.02	97.9
1234678-HpCDF	pg/g	0.00	0.00	0.00	NP
1234789-HpCDF	pg/g	0.00	0.00	0.00	NP
OCDF	pg/g	0.00	0.00	0.00	NP
SUM PCDF	**pg/g**	**4.39**	**0.08**	**0.72**	**97.4**
SUM PCDD/PCDF	**pg/g**	**45.6**	**0.58**	**5.85**	**98.0**
nonotho-PCB (TE, 2005)					
33'44'-TeCB (PCB-77)	pg/g	0.09	0.00	0.01	98.7
344'5-TeCB (PCB-81)	pg/g	0.01	0.00	0.00	100.0
33'44'5-PeCB (PCB-126)	pg/g	9.97	0.15	1.67	97.5
33'44'55'-HCB (PCB-169)	pg/g	0.73	0.02	0.05	98.2
SUM TE-PCB	**pg/g**	**10.8**	**0.16**	**1.73**	**97.6**
PCB's					
PeCB	ng/g	1.09	0.03	0.11	97.8
HCB	ng/g	11.9	0.12	0.94	98.7
2,2',5-TriCB	ng/g	9.72	0.07	0.89	98.7
2,4,4'-TriCB	ng/g	10.3	0.08	0.85	98.7
2,4',5-TriCB	ng/g	10.8	0.09	1.00	98.6
2',3,4-TriCB	ng/g	6.77	0.05	0.57	98.7
3,4,4'-TriCB	ng/g	2.23	0.02	0.18	98.7
Sum TriCB	**ng/g**	**56.4**	**0.39**	**5.00**	**98.7**
2,2'4,4'-TetCB	ng/g	5.8	0.05	0.62	98.4
2,2''5,5'-TetCB	ng/g	17.7	0.15	2.16	98.3
2,3',4,4'-TetCB	ng/g	11.2	0.1	1.14	98.5
2,4,4',5-TetCB	ng/g	6.54	0.06	0.68	98.4
Sum TetraCB	**ng/g**	**53.3**	**0.87**	**10.4**	**97.1**
2,2'4,4',5-PenCB	ng/g	9.83	0.11	1.38	97.9
2,2',4,5,5'-PenCB	ng/g	24.9	0.27	3.81	97.8
2,3,3',4,4'-PenCB	ng/g	6.96	0.08	1.01	97.9
2,3,4,4',5-PenCB	ng/g	0.6	0.01	0.1	97.4
2,3',4,4',5-PenCB	ng/g	17.7	0.21	2.81	97.7
2',3,3',4,5-PenCB	ng/g	0.13	<0.01	0.02	>95.4
2',3,4,4',5-PenCB	ng/g	0.28	<0.01	0.03	>97.5

Sum PentaCB	**ng/g**	**60.4**	**1.03**	**15.6**	**96.3**
2,2',3,3',4,4'-HexCB	ng/g	5.25	0.07	0.95	97.4
2,2',3,4,4',5'-HexCB	ng/g	44.1	0.55	8.21	97.4
2,2',3,5,5'-HexCB	ng/g	6.63	0.08	1.18	97.5
2,2',3,4',5',6-HexCB	ng/g	25.1	0.3	4.43	97.5
2,2',4,4',5,5'-HexCB	ng/g	43.2	0.59	8.46	97.2
2,3,3',4,4',5-HexCB	ng/g	2.59	0.04	0.5	97.2
2,3,3',4,4',5'-HexCB	ng/g	0.45	<0.01	0.08	>97.1
2,3',4,4',5,5'-HexCB	ng/g	1.14	0.02	0.34	95.8
Sum HexaCB	**ng/g**	**128**	**2.21**	**33.7**	**96.3**
2,2',3,3',4,4',5-HepCB	ng/g	8.14	0.13	1.63	97.1
2,2',3,4,4',5,5'-HepCB	ng/g	26.2	0.43	6.05	96.7
2,2',3,4,4',5',6-HepCB	ng/g	4.97	0.07	1.01	97.1
2,2',3,4',5,5',6-HepCB	ng/g	15.1	0.2	3.19	97.0
2,3,3',4,4',5,5'-HepCB	ng/g	0.28	<0.01	0.07	>95.8
Sum HeptaCB	**ng/g**	**54.6**	**1.06**	**16.6**	**95.7**
2,2',3,3',4,4',5,5'-Oct-CB	ng/g	4.69	0.1	1.53	95.4
2,2,'3,3',4,4'5,5',6-NonCB	ng/g	2.17	0.04	0.48	96.7
DecaCB	ng/g	0.39	0.02	0.09	95.4
Sum 7 PCB	**ng/g**	**184**	**2.29**	**32.4**	**97.5**
Sum PCB	**ng/g**	**360**	**5.73**	**83.4**	**96.7**
PBDE's (Polybrominated Diphenyl Ethers)					
TBA	ng/g	2.59	<0.11	0.21	>97.5
2,4,4'-TriBDE	ng/g	0.26	<0.04	0.04	>92.7
2,2',4,4'-TetBDE	ng/g	12.9	0.25	3.5	96.1
2,2',4,5'-TetBDE	ng/g	NP	<0.04	0.63	N/A
2,3',4,4'-TetBDE	ng/g	<0,07	<0.04	0.05	>71.2
2,3',4',6-TetBDE	ng/g	NP	<0.04	<0.01	N/A
2,2',4,5'+2,3',4',6-TetBDE	ng/g	1.96	<0.08	11.3	>30.4
3,3',4,4'-Tet-BDE	ng/g	<0,05	<0.02	<0.01	>83.4
2,2',3,4,4'-PenBDE	ng/g	<0,11	<0.05	<0.02	>81.6
2,2',4,4',5-PenBDE	ng/g	0.67	<0.04	0.26	>93.3
2,2'4,4',6-PenBDE	ng/g	0.92	<0.03	0.34	>94.5
2,3',4,4',6-PenBDE	ng/g	<0,07	<0.05	0.04	>67.8
2,2',3,4,4',5'-HexBDE	ng/g	<0,17	<0.18	<0.02	>60.9
2,2',4,4',5,5'-HexBDE	ng/g	0.29	<0.16	0.11	>75.8
2,2',4,4',5,6'-HexBDE	ng/g	0.72	<0.12	0.28	>89.5
2,2',3,4,4',5',6-HepBDE	ng/g	<0,12	<0.11	<0.01	>66.3
2,2',3,3',4,4',5,6'-OctBDE	ng/g	<0,39	<0.53	<0.08	>49.1
2,2',3,3',4,4',5,5',6-NonBDE	ng/g	0.75	<0.34	0.55	>75.2
DecaBDE	ng/g	6.30	<0.51	6.21	>85.5
Chlorinated Pesticides					
Dieldrin	ng/g	17	<2.96	<3.5	>91.4
Aldrin		<0.02	<0.05	<0.04	NP
Isodrin		<0.04	<0.16	<0.11	NP
Endrin		23.8	<2.44	<2.92	>94.9
Heptachlor-exo-epoxide		<1.37	<2.08	<2.32	>25.9

Heptachlor-endo-epoxide		<0.54	<0.83	<0.93	>24.8
trans-chlordane		0.87	<0.09	0.25	>92.9
cis-chlordane		4.19	<0.24	0.99	>95.2
Oxy-chlordane		1.39	<0.94	<1.50	>63.1
Chlordane		<0.19	<1.04	<1.21	NP
Heptachlor		<0.08	<0.28	<0.31	NP
Endosulfan-I		0.13	<0.07	<0.09	>72.6
Mirex		0.34			NP
PAH's					
Naphtalene	ng/g	11.5	7.86	23.7	51.3
Acenaphtylene	ng/g	1.8	<0.27	0.83	>89.2
Acenaphtene	ng/g	7.13	0.68	2.22	92.9
Fluorene	ng/g	12.8	2.01	7.34	87.6
Phenantrene	ng/g	9.32	5.15	16.5	59.4
Antracene	ng/g	8.36	<0.27	1.1	>97.3
Fluoranthene	ng/g	6.96	0.86	1.65	92.8
Pyrene	ng/g	3.54	3.63	3.03	53.3
Benz(a)anthracene	ng/g	1.03	<0.27	<0.27	>87.6
Chrysene/Triphenylene	ng/g	1.98	<0.27	0.39	>92.8
Benzo(b/j/k)fluoranthenes	ng/g	1.16	<0.27	0.34	>88.2
Benzo(a)pyrene	ng/g	12.3	<0.27	1.58	>97.7
Indeno(1,2,3-cd)pyrene	ng/g	<0,75	<0.50	<0.5	>68.4
Dibenz(az/ah)anthracene	ng/g	<0,75	<0.50	<0.5	>68.4
Benzo(ghi)perylene	ng/g	<0,75	<0.50	<0.5	>68.4
Sum possibly carcenogenic	**ng/g**	**16**	**1.81**	**3.19**	**93.6**
Sum 16 EPA PAH	**ng/g**	**80.1**	**23.3**	**60.5**	**80.7**
HCH (Hexachlorocyclohexane)					
Alpha-HCH	ng/g	1.57	<0.02	0.18	>98.2
Beta-HCH	ng/g	11.2	0.12	2.36	97.1
Gamma-HCH	ng/g	29.0	0.21	3.68	98.2
Delta-HCH	ng/g	<0.45	<1.15	<0.34	NP
DDT					
o,p'-DDE	ng/g	0.75	0.03	0.11	96.8
p,p'-DDE	ng/g	81.9	0.86	13.7	97.6
o,p'-DDD	ng/g	2.56	0.06	0.71	95.9
p,p'-DDD	ng/g	44.8	0.76	16.2	95.1
o,p'-DDT	ng/g	2.99	0.08	0.33	97.7
p,p'-DDT	ng/g	156	2.63	55.7	95.2
Sum DDT	**ng/g**	**289**	**4.41**	**86.8**	95.9

"NP" indicates that the calculation and/or measurement were not performed.

Table 18: Yield and impurities removal in the final product.

Compound	Unit	Feed	Product 1st Stage	Product 2nd Stage	Overall Yield Simulation (%)	Overall Yield (% Yield)

TG	A%	89.7	97.17	89.17	99.1	>99
DG	A%	3.7	1.98	7.99	84.9	89.0
Total	A%	93.4	99.15	97.16	98.5	>99
Dry Weight	g/L	309.2	219.9	73.0	96.1	94.7
Impurity	**Unit**	**Feed**	**Product 1st Stage**	**Product 2nd Stage**	**Overall removal Simulation (%)**	**Overall Removal (%)**
Total cholesterol	mg/g	9.31	1.987	9.875	NP	79.9
Free cholesterol	mg/g	5.83	0.00	2.247	91.5	95.4
Esterified cholesterol	mg/g	3.48	1.987	7.628	NP	53.7
Acid value	mg KOH/g	7.4	0.00	0.00	97.3	100.0
Alpha tocopherol	mg/g	0.098	0.00	0.102	NP	87.7
Vitamin A	mg/g	23.6	5.1	>30	NP	~77.3
Vitamin D3	I.U./kg	32400	8790	44200	NP	74.2

"NP" indicates that the calculation and/or measurement were not performed.
"A%" = area percentage, or the % of total peaks in the GPC chromatogram.

[0215] The separation performance results achieved by the membranes during each stage are described in Table 19a and 19b.

Table 19a: Summary of the yield results during each stage of the diafiltration

Compound	1st Stage Simulated Yield (%)	1st Stage Yield (%)	2nd Stage Simulated Yield (%)	2nd Stage Yield (%)
TG	87.3	77.0	11.8	23.5
DG	50.7	38.1	34.2	51.0
Dry Weight	77.8	71.1	18.3	23.6

Table 19b: Summary of the impurities removal results after the 1st and 2nd stage of the diafiltration and respective simulation.

Impurity	1st Stage Simulation (%)	1st Stage Removal (%)	After 2nd Stage Simulation (%)	After 2nd Stage Removal (%)
Total cholesterol	NP	84.8	NP	74.9
Free cholesterol	98.3	100.0	91.5	90.9
Alpha tocopherol	NP	100.0	NP	75.4
Vitamin A	NP	84.6	NP	70.0
Vitamin D3	NP	80.7	NP	67.8

SUM PCDD	NP	99.1	NP	97.1
Sum 7 PCB	NP	99.1	NP	95.8
2,2',4,4',5,6'-HexBDE	NP	88.1	NP	90.8
DecaBDE	NP	94.2	NP	76.7
Benzo(a)pyrene	NP	98.4	NP	97.0
Sum DDT	NP	98.9	NP	92.9

"NP" indicates that the calculation and/or measurement were not performed.

[0216] The second stage recovered a significant part of the yield lost to the permeate stream during the 1st diafiltration stage. However, operating the 2nd diafiltration increased the quantity of impurities in the final product oil. Nevertheless the product was still a high quality oil relative to the feed oil.

Conclusions

[0217] By applying the DMD process with 6.8 DV, the yield lost in the permeate stream in the 1st stage was recovered, improving the final glycerides yield to >99%. As mentioned above, the free cholesterol rejection was higher than the 1st stage, which led to a removal of about 91% of the free cholesterol. The two-stage diafiltration process demonstrated that a high removal of pollutants and other important impurities and recovery of a high yield of valuable glyceride product is possible, leading to the desired high quality, low impurity oil.

WHAT IS CLAIMED IS:

1. A process for reducing at least one impurity from a marine fatty acid oil mixture comprising:

(a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution;

(b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising the at least one impurity; and

(c) removing the organic solvent from the retentate to form a purified marine oil,

wherein the at least one impurity in the marine oil is reduced compared to the marine fatty acid oil mixture, and the marine fatty acid oil mixture comprises marine oil chosen from triglyceride oils, phospholipid oils, and any combination thereof; and

further wherein the purified marine oil comprises free cholesterol in an amount less than about 2 mg/g, and the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

2. The process according to claim 1, wherein the purified marine oil comprises free cholesterol in an amount ranging from about 0 mg/g to about 1 mg/g.

3. The process according to claim 1, wherein the purified marine oil comprises free cholesterol in an amount ranging from about 0 mg/g to about 0.5 mg/g.

4. The process according to claim 1, wherein the passing of the solution across the at least one selective membrane is chosen from diafiltration, cross-flow filtration, and tangential-flow filtration.

5. The process according to claim 1, further comprising recovering any solvent content from the permeate.

6. The process according to claim 1, further comprising subjecting the permeate to at least one additional processing step.

7. The process according to claim 1, further comprising repeating the process of mixing, passing, and removing to achieve the purified marine oil comprising a predetermined level of the at least one impurity, compared to the marine fatty acid oil mixture.

8. The process according to claim 7, wherein the repeating of the process of mixing, passing, and removing continues for a period of time ranging from about 10 minutes to about twenty hours.

9. The process according to claim 8, wherein the period of time ranges from about 30 minutes to about five hours.

10. The process according to claim 1, further comprising passing the permeate across at least one second selective membrane to form a second retentate comprising oil content and a second permeate comprising at least one impurity,

wherein the at least one second selective membrane may be the same as, or different from, the at least one selective membrane.

11. The process according to claim 10, further comprising repeating the process of mixing, passing, and removing to achieve a permeate comprising a predetermined level of at least one impurity and/or a decreased level of oil content, compared to the marine fatty acid oil mixture.

12. The process according to claim 1, wherein the marine fatty acid oil mixture comprises marine triglyceride oil.

13. The process according to claim 12, wherein the marine triglyceride oil comprises mono-, di-, and triglycerides.

14. The process according to claim 1, wherein the marine oil is chosen from fish oil, shellfish oil, oil from krill or other crustaceans, squid oil, marine mammal oil, marine algae oil, zooplankton oil, marine plant oil, and marine microbial oil, and combinations thereof.

15. The process according to claim 14, wherein the marine oil comprises fish oil.

16. The process according to claim 15, wherein the fish oil is chosen from species of sardine/pilchard, anchovy, herring, capelin, sand eel, menhaden, tuna, mackerel, halibut, and blue whiting.

17. The process according to claim 15, wherein the fish oil comprises liver oil chosen from cod, saithe, pollock, and haddock.

18. The process according to claim 14, wherein the marine oil comprises krill oil.

19. The process according to claim 1, wherein the at least one impurity is chosen from environmental pollutants, cholesterol, monoglycerides, oxidation products, components that create unwanted smell and/or taste in the oil mixture, Vitamin A, Vitamin D, Vitamin E, astaxanthin, canthaxanthin, and other carotenoids.

20. The process according to claim 19, wherein the at least one impurity comprises astaxanthin, other carotenoids, Vitamin E, and Vitamin A.

21. The process according to claim 1, wherein the process produces a reduction in the level of at least one impurity in the purified marine oil ranging from 70% to 100% compared to the marine fatty acid oil mixture.

22. The process according to claim 21, wherein the process produces a reduction in the level of at least one impurity in the purified marine oil ranging from 85% to 99% compared to the marine fatty acid oil mixture.

23. The process according to claim 1, wherein the permeate comprises an increased concentration of at least one component chosen from fat soluble vitamins, cholesterol, astaxanthin, canthaxanthin, and other carotenoids, compared to the marine fatty acid oil mixture.

24. The process according to claim 23, wherein the at least one component is chosen from astaxanthin, other carotenoids, Vitamin E, and Vitamin A.

25. The process according to claim 19, wherein the environmental pollutants are chosen from polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes (HCH), dichlorodiphenyltrichloroethane (DDT), dioxins, furans, and nonortho-PCBs.

26. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.1 ng/g of PBDE 47.

27. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.05 ng/g of PBDE 99.

28. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.05 ng/g of PBDE 100.

29. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.02 ng/g of PBDE 154.

30. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.5 ng/g of PBDE 209.

31. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.1 ng/g of a sum of the concentrations of PBDE 47, PBDE 99, and PBDE 100.

32. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.1 ng/g of a sum of the concentrations of PBDE 28, PBDE 47, PBDE 49, PBDE 71, PBDE 99, PBDE 100, and PBDE 154.

33. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 3 ng/g of a total concentration of PCBs.

34. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 1 ng/g of a sum of the concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180.

35. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 30 ng/g of a sum of the concentrations of non-ortho PCB 77, non-ortho PCB 81, non-ortho PCB 126, and non-ortho PCB 169.

36. The process according to claim 25, wherein the purified marine oil comprises a maximum concentration of about 0.2 pg/g of dioxins, calculated as a sum of the concentrations of PCDD, TE 2005.

37. The process according to claim 25, wherein the process produces a reduction of about 85% to about 99% in dioxins in the purified marine oil compared to the marine fatty acid oil mixture.

38. The process according to claim 1, wherein the organic solvent is chosen from aromatics, alkanes, ketones, glycols, chlorinated solvents, esters, ethers, amines, nitriles, aldehydes, phenols, amides, carboxylic acids, alcohols, furans, and dipolar aprotic solvents, and mixtures thereof and with water.

39. The process according to claim 1, wherein the organic solvent is chosen from toluene, xylene, benzene, styrene, anisole, chlorobenzene, dichlorobenzene, chloroform, dichloromethane, dichloroethane, methyl acetate, ethyl acetate, butyl acetate, methyl ether ketone (MEK), methyl iso butyl ketone (MIBK), acetone, ethylene glycols, ethanol, methanol, isopropanol, butanol, hexane, cyclohexane, heptanes, dimethoxyethane, methyl tert butyl ether (MTBE), diethyl ether, adiponitrile, N,N dimethylformamide, dimethylsulfoxide, N,N dimethylacetamide, dioxane, nitromethane, nitrobenzene, pyridine, carbon disulfide, tetrahydrofuran, methyl-tetrahydrofuran, N-methyl pyrrolidone, N-ethyl pyrrolidone, acetonitrile, and mixtures thereof and with water.

40. The process according to claim 39, wherein the organic solvent is chosen from ethyl acetate, isopropanol (2-propanol), and acetone.

41. The process according to claim 1, wherein the at least one selective membrane comprises a material chosen from polyethylene, polypropylene, polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF), polysulfone, polyethersulfone, polyacrylonitrile, polyamide, polyimide, polyamideimide, polyetherimide,

cellulose acetate, polyaniline, polypyrrole, polyetheretherketone (PEEK), polybenzimidazole, and mixtures thereof.

42. The process according to claim 1, wherein the at least one selective membrane is a composite material comprising a support and a thin, non-porous, selectively permeable layer.

43. The process according to claim 42, wherein the thin, non-porous, selectively permeable layer comprises a material chosen from modified polysiloxane based elastomers including polydimethylsiloxane (PDMS) based elastomers, ethylene-propylene diene (EPDM) based elastomers, polynorbornene based elastomers, polyoctenamer based elastomers, polyurethane based elastomers, butadiene and nitrile butadiene rubber based elastomers, natural rubber, butyl rubber based elastomers, polychloroprene (Neoprene) based elastomers, epichlorohydrin elastomers, polyacrylate elastomers, polyethylene, polypropylene, polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF) based elastomers, polyetherblock amides (PEBAX), polyurethane elastomers, crosslinked polyether, polyamide, polyaniline, polypyrrole, and mixtures thereof.

44. The process according to claim 1, wherein the at least one selective membrane comprises an inorganic material chosen from silicon carbide, silicon oxide, zirconium oxide, titanium oxide, and zeolites.

45. The process according to claim 1, wherein the at least one selective membrane comprises a polymer membrane with dispersed organic or inorganic matrices in the form of powdered solids present in amounts up to about 20 wt% of the polymer membrane.

46. The process according to claim 45, wherein the dispersed organic matrices comprise carbon molecular sieve matrices.

47. The process according to claim 45, wherein the dispersed inorganic matrices comprise a material chosen from zeolites, metal oxides, and mixed metal oxides.

48. The process according to claim 47, wherein the metal oxides are chosen from titanium dioxide, zinc oxide, and silicon dioxide.

49. The process according to claim 47, wherein the mixed metal oxides are chosen from mixtures of cerium, zirconium, and magnesium may also be used.

50. The process according to claim 45, wherein the dispersed organic or inorganic matrices comprise particles of less than about 1.0 micron in diameter.

51. The process according to claim 50, wherein the dispersed organic or inorganic matrices comprise particles of less than about 0.1 microns in diameter.

52. The process according to claim 51, where in the dispersed organic or inorganic matrices comprise particles of less than about 0.01 microns in diameter.

53. The process according to claim 1, wherein the at least one selective membrane comprises two membranes.

54. The process according to claim 1, wherein the at least one selective membrane comprises three membranes.

55. The process according to claim 1, wherein the at least one selective membrane comprises a nanofiltration membrane.

56. The process according to claim 1, wherein the at least one selective membrane comprises an ultrafiltration membrane.

57. The process according to claim 1, wherein the at least one selective membrane has a molecular weight cut-off ranging from about 150 g/mol to about 1,500 g/mol.

58. The process according to claim 57, wherein the at least one selective membrane has a molecular weight cut-off ranging from about 200 g/mol to about 700 g/mol.

59. The process according to claim 1, wherein the purified marine oil comprises:

- a lower level of BDE-47 (2,2',4,4'- tetrabromodiphenyl ether) compared to the marine fatty acid oil mixture.

60. The process according to claim 1, wherein the permeate comprises at least one of cholesterol, hormones, Vitamin A, Vitamin D, and Vitamin E, with an increased concentration compared to the marine fatty acid oil mixture.

61. The process according to claim 1, wherein the marine fatty acid oil mixture comprises greater than about 90% triglyceride or phospholipid oils.

62. The process according to 1, wherein the marine fatty acid oil mixture comprises at least from about 10% to about 30% by weight of omega-3 fatty acids.

63. The process according to claim 1, wherein the marine fatty acid oil mixture comprises PBDE 47 in a concentration ranging from about 0.1 ng/g to about 5 ng/g.

64. The process according to claim 1, wherein the marine fatty acid oil mixture comprises PBDE 99 in a concentration ranging from about 0.05 ng/g to about 5 ng/g.

65. The process according to claim 1, wherein the marine fatty acid oil mixture comprises PBDE 100 in a concentration ranging from about 0.05 ng/g to about 5 ng/g.

66. The process according to claim 1, wherein the marine fatty acid oil mixture comprises PBDE 209 in a concentration ranging from about 0.05 ng/g to about 5 ng/g.

67. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a sum of concentrations of PBDE 47, PBDE 99, and PBDE 100 ranging from about 0.1 ng/g to about 10 ng/g.

68. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a sum of concentrations of PBDE 28, PBDE 47, PBDE 49, PBDE 71, PBDE 99, PBDE 100, and PBDE 154 ranging from about 0.2 ng/g to about 20 ng/g.

69. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a total PCB concentration ranging from about 5 ng/g to about 1,000 ng/g.

70. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a sum of concentrations of PCB 28, PCB 52, PCB 101, PCB 105, PCB 118, PCB 138, PCB 153, and PCB 180 ranging from about 2 ng/g to about 300 ng/g.

71. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a sum of concentrations of non-ortho PCB 77, non-ortho PCB 81, non-ortho PCB 126, and non-ortho PCB 169 ranging from about 20 pg/g to about 1,700 pg/g.

72. The process according to claim 1, wherein the marine fatty acid oil mixture comprises a sum of concentrations of dioxins (sum PCDD, TE 2005) ranging from about 0.2 pg/g to about 20 pg/g.

73. The process according to claim 1, wherein the process is performed at a temperature ranging from about -10 °C to about 60 °C.

74. The process according to claim 73, wherein the process is performed at a temperature ranging from about 25 °C to about 50 °C.

75. The process according to claim 1, wherein the solution is passed across the at least one selective membrane at a filtration pressure ranging from about 5 bar to about 70 bar.

76. The process according to claim 75, wherein the filtration pressure ranges from about 15 bar to about 60 bar.

77. The process according to claim 1, wherein the solution is passed across the at least one selective membrane via tangential flow filtration.

78. The process according to claim 77, wherein the tangential flow filtration comprises a linear velocity ranging from about 0.1 m/s to about 5 m/s.

79. The process according to claim 78, wherein the linear velocity ranges from about 0.5 m/s to about 3 m/s.

80. A process for making a marine concentrate comprising at least one natural component from a marine fatty acid oil mixture, comprising:

(a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution;

(b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content and a permeate forms comprising the at least one natural component; and

(c) removing the organic solvent from the permeate to form a marine concentrate comprising the at least one natural component,

wherein the at least one natural component is chosen from fat soluble vitamins, cholesterol, astaxanthin, canthaxanthin, and other carotenoids, and the marine fatty acid oil mixture comprises marine oil chosen from triglyceride oils, phospholipid oils, and any combination thereof; and

further wherein the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one natural component, R_{Des}.

81. The process according to claim 80, wherein the at least one natural component is chosen from Vitamin A, Vitamin D, Vitamin E, and astaxanthin.

82. The process according to claim 80, further comprising passing the permeate across at least one second selective membrane to form a second retentate comprising oil content and a second permeate comprising at least one natural component,

wherein the at least one second selective membrane may be the same as, or different from, the at least one selective membrane.

83. The process according to claim 82, further comprising repeating the process of mixing, passing, and removing to achieve a decreased level of oil content, compared to the marine fatty acid oil mixture.

84. The process according to claim 80, further comprising purifying the marine concentrate using a method chosen from HPLC, supercritical fluid chromatography, distillation, molecular distillation, short path evaporation, thin film evaporation, extraction, absorption, and any combination thereof.

85. A purified marine oil comprising:

- less than about 2 mg/g total cholesterol; and
- an environmental pollutant level comprising:
  - a maximum concentration of about 1 ng/g for the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180;
  - a maximum concentration of about 0.1 ng/g for the sum of PBDE congener nos. 28, 47, 49, 71, 99, 100, and 154;
  - a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g for decabromodiphenyl ether; and
  - a maximum concentration of 1.0 pg/g for the sum of PCDD, TE 2005.

86. The purified marine oil according to claim 85, wherein the maximum concentration for decabromodiphenyl ether is about 0.5 ng/g.

87. The purified marine oil according to claim 86, wherein the maximum concentration for decabromodiphenyl ether is about 0.2 ng/g.

88. The purified marine oil according to claim 85, wherein the decabromodiphenyl ether is PBDE-47 (2,2',4,4'- tetrabromodiphenyl ether).

89. The purified marine oil according to claim 88, wherein the maximum concentration of PBDE-47 (2,2',4,4'- tetrabromodiphenyl ether) is about 0.1 ng/g.

90. The purified marine oil according to claim 85, wherein the maximum concentration of PBDE 100 is about 0.01 ng/g.

91. The purified marine oil according to claim 85, wherein the maximum concentration of PBDE 99 is about 0.05 ng/g.

92. The purified marine oil according to claim 85, wherein the maximum concentration of PBDE 154 is about 0.02 ng/g.

93. The purified marine oil according to claim 85, wherein the maximum concentration of PBDE 209 is about 0.1 ng/g.

94. A purified marine oil comprising greater than about 90% triglyceride oil from marine origin comprising:

- less than about 2 mg/g total cholesterol; and
- an environmental pollutant level comprising a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g decabromodiphenyl ether.

95. A purified marine oil comprising greater than about 90% triglyceride oil from marine origin comprising

- less than about 0.3 mg/g free cholesterol; and

- an environmental pollutant level comprising a maximum concentration ranging from about 0.1 ng/g to about 1.0 ng/g decabromodiphenyl ether.

96. A process for reducing at least one impurity from a marine triglyceride oil comprising:

(a) mixing the marine triglyceride oil with an organic solvent to form a solution, wherein the organic solvent is chosen from ethyl acetate, isopropanol, and acetone;

(b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising the at least one impurity; and

(c) removing the organic solvent from the retentate to form a purified marine oil,

wherein the marine triglyceride oil comprises marine oil comprising mono-, di- and triglycerides and the process is performed at a temperature ranging from 30 °C to 50 °C; and

further wherein the purified marine oil comprises free cholesterol in an amount less than about 1 mg/g and comprises about 0.1 ng/g of PBDE 47 (2,2',4,4'- tetrabromodiphenyl ether); and

further wherein the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}, and has a molecular weight cut-off ranging from about 200 g/mol to about 700 g/mol.

97. A process for reducing at least one impurity from a marine triglyceride oil comprising:

(a) mixing the marine triglyceride oil with an organic solvent to form a solution;

(b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising the at least one impurity;

(c) removing the organic solvent from the retentate to form a purified marine oil;

(d) subjecting the purified marine oil to transesterification to form a second purified marine oil comprising fatty acids at least 90% in ethyl ester form; and

(e) subjecting the second purified marine oil to short path evaporation, molecular distillation, thin film evaporation, or distillation to form a third purified marine oil (in ethyl ester form);

wherein the at least one impurity in the third purified marine oil is reduced compared to the marine triglyceride oil, and the marine triglyceride oil comprises marine oil comprising mono-, di- and triglycerides; and

further wherein the third purified marine oil comprises total cholesterol in an amount less than about 0.5 mg/g, and

further wherein the at least one selective membrane has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

98. A purified marine oil comprising:

at least 40% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), by weight of the purified marine oil, wherein the EPA and DHA are in ethyl ester form; and

total cholesterol in an amount less than about 0.5 mg/g.

99. A process for reducing at least one impurity from a marine fatty acid oil mixture comprising:

(a) mixing the marine fatty acid oil mixture with an organic solvent to form a solution;

(b) passing the solution across at least one selective membrane, wherein a retentate forms comprising an oil content, and a permeate forms comprising at least one impurity;

(c) removing the organic solvent from the retentate to form a purified marine oil, and

(d) subjecting the purified marine oil to at least one additional processing step to form a further purified marine oil,

wherein the at least one impurity in the purified marine oil is reduced compared to the marine fatty acid oil mixture, and the marine fatty acid oil mixture comprises marine oil chosen from triglyceride oils, phospholipid oils, and any combination thereof; and

further wherein the further purified marine oil comprises total cholesterol in an amount of less than about 0.5 mg/g; and

further wherein the at least one selective membrane is chosen such that it has a membrane rejection of the oil content, R_{TG}, greater in value than a membrane rejection of the at least one impurity, R_{IMP}.

100. The process according to claim 99, wherein the at least one additional processing step comprises at least one up-concentrating step.

101. The process according to claim 100, wherein the at least one up-concentrating step comprises molecular distillation.

102. The process according to claim **Error! Reference source not found.**, wherein the marine fatty acid oil mixture is present in ester form chosen from ethyl ester and methyl ester form.

103. The process according to claim 99, wherein the further purified marine oil comprises an ethyl ester concentrate or an ethyl ester intermediate product.

104. The process according to claim 99, wherein the further purified marine oil comprises eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present in an amount ranging from about 50% to 80% by weight, relative to the total weight of the further purified marine oil.

105. The process according to claim 99, wherein the further purified marine oil is converted to triglyceride form.

106. The process according to claim 99, wherein the at least one selective membrane comprises silicon rubber.

107. The purified marine oil according to claim 94, further comprising a maximum concentration of 0.015 mg/kg for the sum of PCB congener nos. 28, 52, 101, 118, 138, 153, and 180, and a maximum concentration of about 2 ng/g for the sum of PBDE congener nos. 28, 47, 49, 99, 100, 153, and 154.

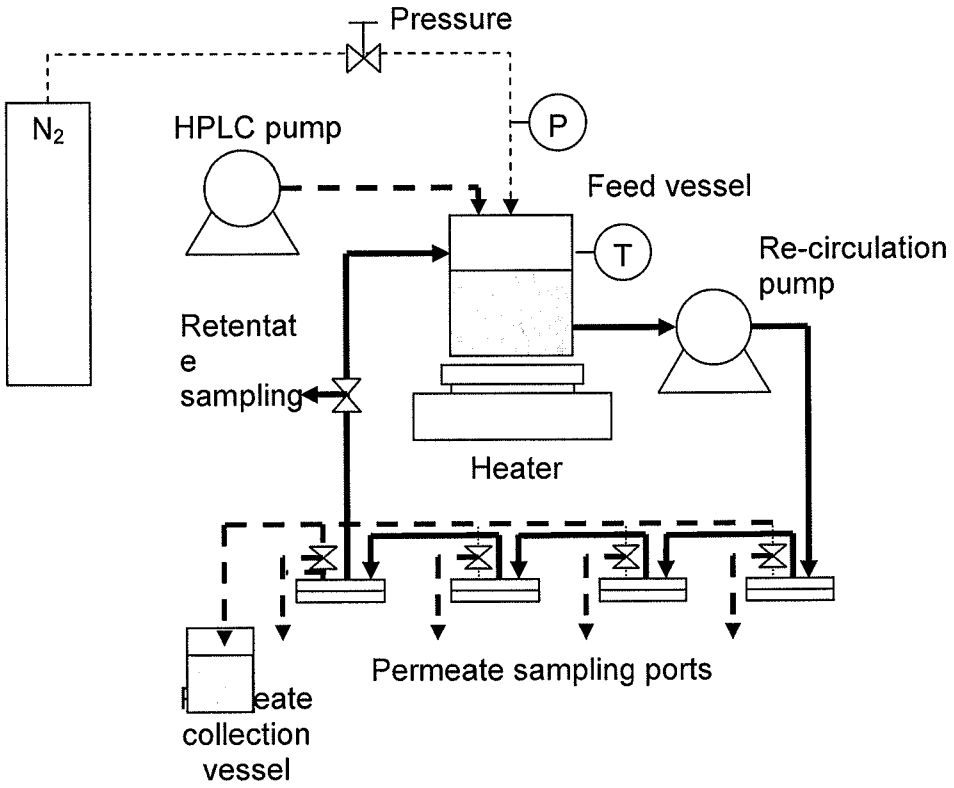
Pressure
N_2
HPLC pump
P
Feed vessel
T
Re-circulation pump
Retentat e sampling
Heater
Permeate sampling ports
collection vessel

FIG. 1

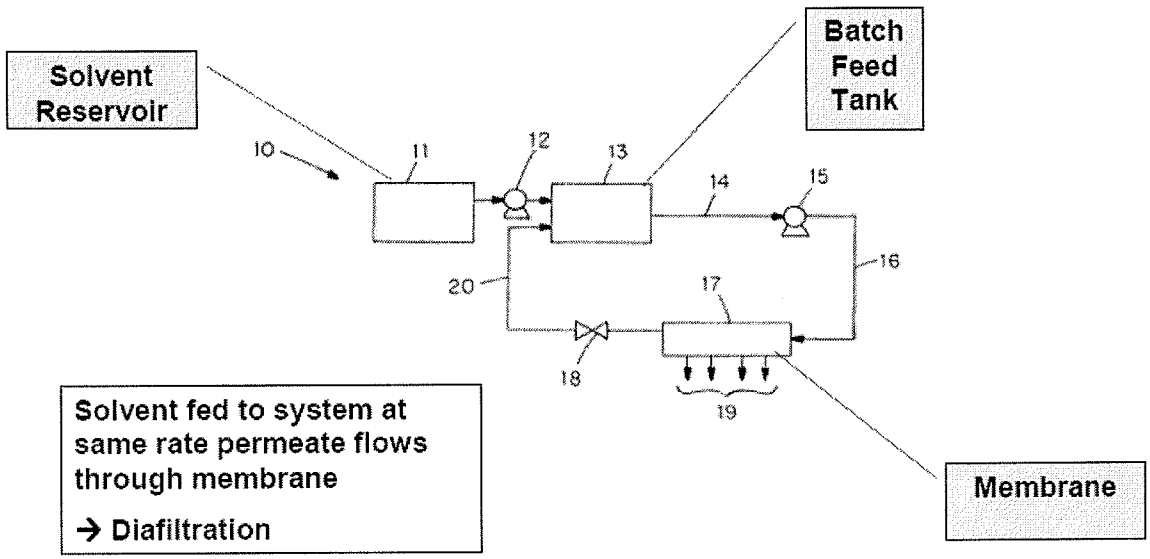
Solvent Reservoir
Batch Feed Tank
10
11
12
13
14
15
16
17
18
19
20
Solvent fed to system at same rate permeate flows through membrane
→ Diafiltration
Membrane

FIG. 2

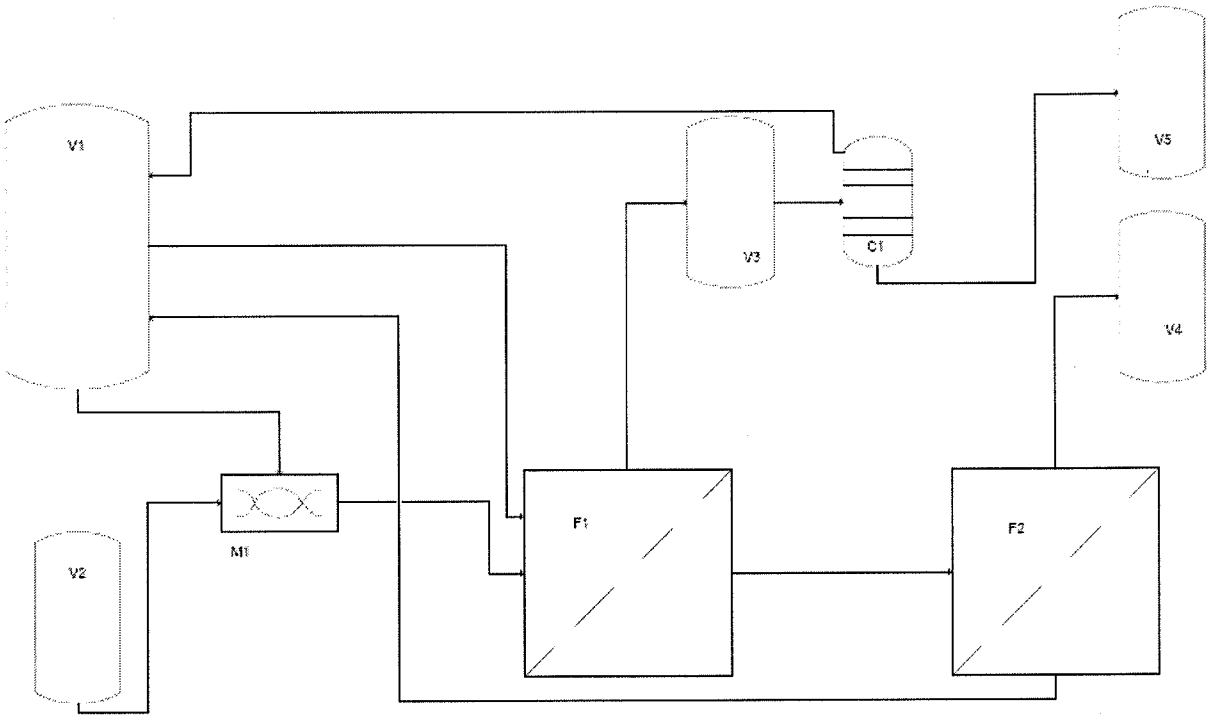

FIG. 3

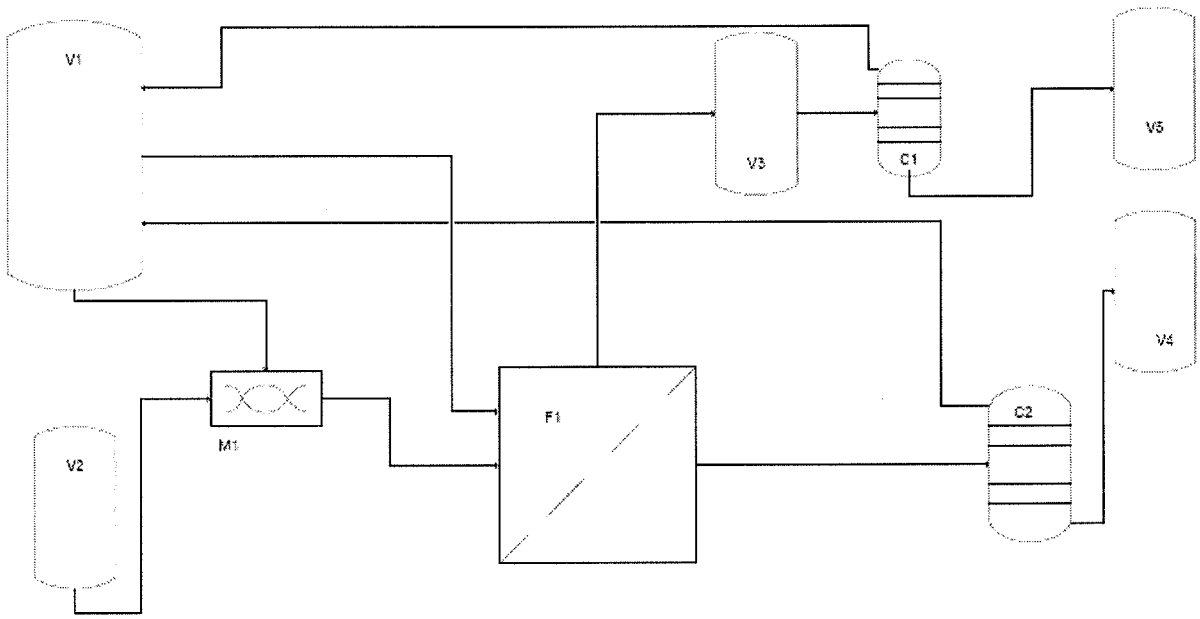

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/002840

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23D9/04 C11B3/00 B01D61/14 C11B3/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23D C11B B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/263886 A1 (KALE ANIKET [US]) 27 October 2011 (2011-10-27) paragraphs [0178] - [0182]; claims 1-3	1-107
X	GB 660 017 A (UNILEVER LTD) 31 October 1951 (1951-10-31) example 3	1-107

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2013	27/03/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Saettel, Damien

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/002840

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	ALLEGRE C ET AL: "Cholesterol removal by nanofiltration: Applications in nutraceutics and nutritional supplements", JOURNAL OF MEMBRANE SCIENCE, ELSEVIER SCIENTIFIC PUBL.COMPANY. AMSTERDAM, NL, vol. 269, no. 1-2, 1 February 2006 (2006-02-01), pages 109-117, XP024931306, ISSN: 0376-7388, DOI: 10.1016/J.MEMSCI.2005.06.025 [retrieved on 2006-02-01] cited in the application page 111, left-hand column, paragraph 3 - page 117, left-hand column, paragraph 1	1-107
A	WO 2008/054228 A1 (DUE MILJOE AS [NO]; BERGE JEAN-PASCAL [FR]; DELANNOY CHARLES [FR]; DHA) 8 May 2008 (2008-05-08) claims; examples	1-107
A	EP 2 295 529 A2 (PRONOVA BIOPHARMA NORGE AS [NO]) 16 March 2011 (2011-03-16) claims; figure 2; examples	1-107

INTERNATIONAL SEARCH REPORT

Information on patent family members

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