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(54) Enrichment of microbial oils

(57) A process for treating a microbial oil, comprising one or more polyunsaturated fatty acids (PUFAs) is disclosed. The process involves adding a precipitate inducer to the microbial oil, cooling the oil and solvent mixture until part of the oil solidifies (to form a precipitate) and then removing the precipitate. Thus a 2-phase system forms consisting of a top layer (liquid, residual oil) and a bottom layer (solid, precipitate). The precipitate contains crystals and once this has been separated from the residual oil then the inducer is removed from the oil. The top layer is thus enriched in the PUFA, while the bottom layer is depleted of the PUFA. Suitable PUFAs include C18, C20 and C22, Ω3 and Ω6 fatty acids and the inducer can be a clear organic liquid such as hexane or acetone.

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Description

[0001] The present invention relates to the treatment of microbial oils, in particular those containing one or more polyunsaturated fatty acids (PUFAs). The treatment comprises adding a solvent to the oil, and cooling the oil until a precipitate forms, and then removing the precipitate. The resulting oil may be enriched in PUFAs.

[0002] Although microbial oils, for example containing PUFAs, are known, there is a need to improve the quality of the oil, and in particular to increase the amount of PUFAs in the oil. The oil can thus be made more concentrated, and so less oil may be required in order to deliver a desired quantity of a PUFA. There is also a need to be able to purify microbial and PUFA-containing oils sufficiently in order that they can be incorporated into foodstuffs such as infant formula or other edible compositions, such as pharmaceuticals. This purification process is desirably efficient and cost-effective.

[0003] WO-A-97/43362 (Gist-Brocades B.V.) describes the extraction of sterols from microbial oils with a polar solvent. However, unlike the present invention, it does not include the cooling of the oil (winterisation) or the formation of precipitate, which is then removed.

[0004] A first aspect of the present invention thus relates to a process for treating a microbial oil or an oil comprising an (e.g. Ω3 or Ω6) PUFA. This process may comprise adding a precipitate inducer (or enhancer) to the oil. Cooling (of the oil and precipitate inducer mixture) can then take place until a precipitate forms or at least part of the oil solidifies. The precipitate (solid or solidified matter) may then be removed (from the e.g. residual oil).

PUFAs and oils

[0005] Preferably the oil is a microbial oil or it comprises one or more PUFAs. It is usually a liquid. Preferred PUFAs are C18, C20 or C22 (e.g. Ω6 or Ω3) PUFAs. Preferred Ω3 and Ω6 PUFAs include:

(Ω3) docosahexaenoic acid (DHA), suitably from algae or fungi, such as the dinoflagellate *Crypthecodinium* or the fungus *Thraustochytrium*;
(Ω6) γ-linolenic acid (GLA);
(Ω3) α-linolenic acid (ALA);
(Ω6) dihomo-γ-linolenic acid (DGLA);
(Ω6) arachidonic acid (ARA); and
(Ω3) eicosapentaenoic acid (EPA).

[0006] The microbial oil may thus comprise an Ω3 or an Ω6 PUFA. The Ω3 PUFA (e.g. DHA)-containing oil may be a marine, e.g. fish (such as tuna) oil. The Ω6 and/or Ω3 PUFA (e.g. ARA, DHA or EPA)-containing oil can be a microbial or single cell oil.

[0007] An Ω6 and/or Ω3 PUFA-containing microbial oil (e.g. GLA, ARA and EPA) can be obtained from fungi, such as *Mortierella*, *Pythium* or *Entomophthora*. Ω3 PUFAs (e.g. EPA) can be produced from algae such as *Porphyridium* or *Nitzschia*.

[0008] Preferably the microbial (or Ω6 or Ω3 (e.g. ARA, DHA or EPA)) oil can be produced by a single cell or a microorganism. This may be a bacteria, yeast, algae or fungi. Preferred fungi are of the order *Mucorales*. The fungus may be of the genus *Mortierella*, *Phycomyces*, *Blakeslea* or *Aspergillus*. Preferred fungi are of the species *Mortierella alpina*, *Blakeslea trispora* and *Aspergillus terreus.*

[0009] Preferred yeasts are of the genus *Pichia* or *Saccharomyces*, for example *Pichia ciferrii*. Bacteria can be of the genus *Propionibacterium.* Preferred algae are dinoflagellate and/or belong to the genus *Crypthecodinium,* for example are of the species *Crypthecodinium cohnii.*

[0010] The Ω6 and/or Ω3 PUFA-containing oil may be an edible oil or a vegetable oil. These include blackcurrant, borage and primrose oils, and often contain an Ω6 PUFA, e.g. GLA. They also include olive, sunflower and soybean, soy flower oils, for example cooking and/or salad oils.

Production of crude oils

[0011] The starting (e.g. crude) oil may be a microbial (e.g. single cell) oil, or it may be a marine (e.g. fish) oil or vegetable oil (either crude or partially treated). In particular, crude oils containing Ω3 PUFAs (DHA and/or EPA) can be marine oils. If the PUFA oil is to contain GLA, then the crude oil may be a vegetable oil, for example blackcurrant, borage, sunflower, soybean or primrose oil.

[0012] A number of documents describe the production of crude PUFA oils. Microbial oils containing ARA are disclosed in WO-A-92/13086 (Martek), EPA in WO-A-91/14427 (Martek) and DHA in WO-A-91/11918 (Martek). The present Applicant has already described various methods for extracting PUFA oils from microbial sources, and these

can be found in WO-A-97/36996 and WO-A-97/37032 (both Gist-Brocades). Preparation of ARA, DHA and EPA-containing oils is also described in WO-A-92/12711 (Martek).

In the oil, it is preferred that most of the PUFA is in the form of triglycerides. Thus, preferably at least 50%, such as at least 60%, or optimally at least 70%, of the PUFA is in triglyceride form. Of these triglycerides, preferably at least 40%, such as at least 50%, and optimally at least 60% of the PUFA is present at the α-position of the glycerol (present in the triglyceride backbone), also known as the 1 or 3 position. It may be preferred that at least 20%, such as at least 30%, optimally at least 40% of the PUFA is at the β(2) position.

[0013] Preferably the original microbial oil is a crude oil. It may have been extracted from microbes or single cells, for example by using a solvent, such as supercritical carbon dioxide, hexane or isopropanol. The oil may have been processed and/or treated before the process of the present invention is performed.

Precipitate inducer

[0014] The inducer which is added to the microbial oil is preferably one that elevates or increases the temperature at which the precipitate forms, for example the temperature at which crystallisation starts. It may thus increase the crystallisation temperature of the oil. The inducer may facilitate precipitation: without the inducer it has not been found possible to form a precipitate (at least at temperatures down to about -20°C, such as those found in freezers). With no inducer added, no precipitate formed in a control experiment (see Comparative Example 5).

[0015] The inducer is preferably an (organic) liquid, although it may be polar or nonpolar. Preferably the inducer will have a melting point of below the precipate, that is to say a temperature bow that at which the precipitate forms. Thus the inducer preferably has an m.p. below -70°C, such as below -80°C optimally below -90°C. The inducer is suitably a clear liquid.

[0016] Suitably the inducer will be a solvent for (or miscible with) fats or oils, in particular a solvent for (or miscible with) saturated triglycerides (that is to say, triglycerides from saturated fatty acids) or a PUFA. Particularly preferred inducers are hexane and/or acetone.

[0017] The inducer is preferably a liquid that is miscible with the oil, and hence a preferred inducer is one that can dissolve or is miscible with a PUFA. Inducers that may thus not be suitable are those that are immiscible with the oils, in particular alcohols (e.g. methanol, isopropanol).

[0018] The precipitate inducer can therefore be regarded as a solvent for the oil to which it is added. However, the inducer is preferably a non-solvent for at least one component of the oil that is present in the precipitate, that is to say it does not dissolve or it is immiscible with this component. This component which may be a single compound) is one that is desired to be removed, and this is achieved by removing the precipitate (or solidified matter) from the remaining oil. Although not wishing to be bound by theory, it is thought that the precipitate inducer may bind to or surround the component that is to be removed. The inducer may thus in same way face the component out of solution (ie. out of the oil). However, whatever the mechanism, the precipitate inducer clearly seems to play an important role in being able to facilitate the formation of the precipitate. It appears to assist or induce precipitate formation, and such a precipitate can contain at least one component that the inducer is not a solvent for.

[0019] In the same way as one can describe compounds as hydrophilic and hydrophobic, one can think of various components in the oil as being either inducer-liking (the inducer is a solvent for that component) or inducer-hating (the inducer is a non-solvent for that component). Once the precipitate performs, the precipitate may contain more inducer-hating component(s) than the remaining oil. Thus, when the oil separates into two phases, namely the remaining oil (usually on top) and the precipitate (usually on the bottom), the oil will be enriched in an inducer-loving component (or depleted in an inducer-hating component) while the precipitate will be enriched in an inducer-hating component (but depleted in an inducer-loving component).

[0020] The ratio of oil:inducer is preferably from 1:1 to 1:10, such as from 1:2 to 1:9. These ratios are particularly applicable if the solvent comprises acetone or hexane.

[0021] The inducer may be added to the oil when one or both of the oil and inducer are liquids, such as at room temperature. However, addition can take place at any suitable temperature of from 0-20°C, preferably from 0°C to 20°C, optimally 2°C to 30°C.

Cooling

[0022] The oil and inducer are mixed and then the mixture is allowed to cool. The mixture is suitably homogeneous, i.e. a one-phase mixture. Cooling may take place naturally or passively (for example by placing the mixture outside in a cool environment). However, the oil can be actively cooled, for example using a heat exchanger.

[0023] Preferably however the oil is cooled by using a refrigerator or freezer. Cooling may take place slowly. Preferably the oil and inducer mixture is placed in an environment that is at a temperature below which a precipitate forms. This temperature is preferably below 0°C or -5°C, such as below -10°C, suitably below - 20°C, and optimally at or

below -25°C. The time taken to cool the oil (and solvent mixture) may be from 1 to 30 hours, such as from 16 to 24 hours, optimally from 18 to 22 hours.

Nature of precipitate (or sediment) and its removal

[0024] The precipitate is usually a solid that forms due to cooling and for convenience this term (including its use in "precipitate inducer") refers to the solidified matter resulting from the lowering in temperature. Preferably the precipitate comprises crystals. If so, then the inducer may increase the crystallisation temperature of the oil (or one or more component(s) in the oil). The crystals may comprise only one component or compound. The precipitate may thus comprise one or more impurities and/or unwanted compounds, for example a saturated triglyceride. Solidification occurs at (or, to put another way, the melting point of the solidified matter or precipitate is) preferably from -1°C to -25°C, such as from -3°C to -20°C, preferably from -5°C to -17°C. Solidification occurs at about - 15°C if the solvent is acetone and about -5°C if the solvent is hexane.

[0025] Suitably the melting point of the precipitate is increased by the addition of the inducer. With no inducer present, no precipitate was formed even when the oil was cooled to -20°C (see Comparative Example 5).

[0026] Preferably the precipitate will not contain much PUFA, or at least only a small amount. The amount of PUFA (e.g. ARA) in the precipitate is preferably less than 40%, such as less than 35%, optimally less than 30% (by weight: 1% here is equivalent to 10g PUFA/kg oil).

[0027] The precipitate is preferably denser than the oil. If this is so, then the precipitate may fall or migrate to the bottom of the oil. It may thus be or form a sediment. In this case the precipitate may be removed by centrifugation. This can take place in a closed system, and so may minimise exposure of the oil to degrading substances, such as oxygen in the atmosphere. Preferred centrifuges are laboratory or industrial centrifuges. Centrifugation may take place at from 2000 rpm to 8000 rpm, such as from 3000 to 7000 rpm, optimally from 4000 to 6000 rpm. Put another way, centrifugation may occur at from 2,000-8,000g, such as from 3,000-7,000g, optimally from 4,000-6,000g. This may take place at from 1 to 4 minutes, such as from 12 to 3 minutes, preferably for about 2 minutes.

[0028] Preferably the precipitate (or crystals) is removed by filtration. Here any crystals formed may be sufficiently large enough to be removed by filtration. One can use a plate and frame filter press or vacuum filtration. If using a filter press, the pressure used is suitably from 0.2 to 0.5 bar. Removal may be by centrifugation or filtration or both.

[0029] The cooling may thus result in a 2-phase system. A top layer may be liquid, such as a residual oil. The bottom layer may be solid, and is thus the solidified matter (or precipitate/sediment). Preferably the top layer is enriched in a PUFA while the bottom layer is depleted in the PUFA. Thus, the residual oil may have a concentration of the PUFA greater than the original oil and the solidified matter a reduced concentration of the PUFA. This was unexpected as PUFAs often have a low m.p. and would be expected to solidify first.

[0030] The residual oil may have a concentration of a PUFA (e.g. ARA) of at least 35%, such as at least 37%, preferably at least 40%, optimally at least 42%.

[0031] Preferably, after the precipitate has been removed, the inducer is removed.

[0032] The inducer may be allowed to evaporate, for example at room temperature or above. A suitable temperature is from 20 to 80 or 100°C, such as from 25 to 60°C, optimally from 30-50°C. Removal of the inducer may take place with the aid of a vacuum.

[0033] As will be realised, since solidification usually takes place below 0°C, then warming to room temperature may cause the solidified matter to melt and once again to become liquid. Thus removal of the solidified matter preferably occurs while that matter is still solid (or at a temperature below the melting point of that matter). Thus removal preferably occurs at below 0°C, preferably below -5°C, optimally below -10°C.

[0034] Steps (b) - cooling - and (c) - removal of the solidified matter - can be repeated at least twice, for example to enrich the oil further each time. Of course all the steps (a) to (c) can be repeated, for example with the same or different inducer.

[0035] The (treated) oil resulting from the process of the invention, which forms the second aspect of the invention, can be used for various purposes without further processing, or can be additionally subjected to one or more purifying and/or refining steps. The oil may thus be subjected to the purifying process described in European patent application no. 00306606.5 filed on 2 August 2000.

[0036] The oil can be used as an additive or a supplement, for example in food compositions, such as in infant formula. It may however also be used in cosmetic or pharmaceutical compositions. The invention in a third aspect therefore relates to an edible composition, such as a foodstuff, feed, pharmaceutical or cosmetic composition which comprises, or to which has been added, the oil of the second aspect of the invention. Preferred compositions are foods such as infant formula and nutritional supplements.

[0037] The oil of the invention can therefore have a relatively high PUFA content. This may be at least 38%, preferably at least 40%, optimally at least 42% (by weight).

[0038] The oil is particularly suitable for nutritional purposes, and so can be used as or in a nutritional supplement.

The oil may be supplied as an oil, or it may be encapsulated, for example, in a gelatin capsule. The oil can thus be incorporated into foods, feeds or foodstuffs, suitable for animal or human consumption. Suitable examples are health drinks and bread.

[0039] Preferred features and characteristics of one aspect of the invention are equally applicable to another aspect *mutatis mutandis*.

[0040] The invention will now be described, by way of example, with reference to the following Examples. These are provided merely for means of illustration, and are not to be construed being limiting on the invention.

EXAMPLE 1

[0041] Crude arachidonic acid (ARA) oil (100 ml) was obtained from *Mortierella alpina* using hexane as the extracting solvent. The protocol for preparing such an oil is described in Example 16 of WO-A-97/36996 (Gist-Brocades B.V.). This crude oil was mixed with 400 ml of n-hexane. The resulting homogeneous mixture was placed in a freezer held at a temperature of from -18°C to -25°C for 20 hours. A precipitate of crystals formed at about -15°C. Hence a 2-phase system formed, with a top (liquid) layer (the remaining oil) and a bottom layer (solid, precipitate). The precipitate was separated using a laboratory centrifuge (type Sigma 4-10) at 5000 rpm for 2 minutes. The hexane was removed from each of the remaining oil and the precipitate by evaporation overnight at 30°C in a vacuum. The ARA content of the precipitate and residual oil was analyzed by means of gas chromatography (GC) and the results are shown below in Table 1.

Table 1

Substance	ARA (g/kg)
Original oil	345
Oil:precipitate	317
Oil:residual oil	406

EXAMPLE 2

[0042] Crude ARA oil (20 ml), from the same source as that in Example 1, was mixed with 80 ml of acetone. The resulting homogeneous mixture was placed in a freezer held at a temperature of from -18°C to -25°C for 20 hours. A precipitate of crystals formed at about -15°C to give a 2-phase system consisting of a top liquid layer and a bottom solid layer. The precipitate was separated using a laboratory centrifuge (type Sigma 4-10) at 5000 rpm for 2 minutes. The hexane was removed separately from both the residual oil and precipitate by evaporation overnight at 30°C in a vacuum. The ARA content of both layers was analyzed by means of gas chromatography (GC) and the results are shown below in Table 2.

Table 2

Substance	ARA (g/kg)
Original oil	345
Oil:precipitate	240
Oil:residual oil	433

EXAMPLE 3

[0043] Different amounts of solvent (acetone) were added to the crude arachidonic oil used in Example 1. The resulting homogeneous mixtures were placed in a freezer held at a temperature of from -18°C to -25°C for 20 hours. A precipitate of crystals formed at about -15°C to give a 2-phase system of a top layer (liquid) and a bottom layer (solid, precipitate). The precipitate was separated using a laboratory centrifuge (type Sigma 4-10) at 5000 rpm for 2 minutes. The hexane from both layers was removed by evaporation overnight at 30°C in a vacuum and their ARA content was analyzed by means of gas chromatography (GC) The results are shown below in Table 3.

Table 3

Ratio of Oil:acetone	ARA (g/kg)	
	Oil:top layer (residual oil)	Oil:bottom layer (precipitate)
Original oil	358	
1:1	364	351
1:2	399	326
1:3	394	296
1:4	421	261

EXAMPLE 4

[0044] Different amounts of solvent (acetone) were added to the crude arachidonic oil used in Example 1. The resulting homogeneous mixtures were placed in a freezer held at a temperature of from -18°C to -25°C for 20 hours. A precipitate of crystals formed at about -15°C to give a 2-phase system consisting of a top layer (liquid, residual oil) and a bottom layer (solid, precipitate). The precipitate was separated using a laboratory centrifuge (type Sigma 4-10) at 5000 rpm for 2 minutes. The acetone was removed from both layers separately by evaporation overnight at 30°C in a vacuum. The ARA content of both layers was analyzed by means of gas chromatography (GC) and the results are shown below in Table 4.

Table 4

Ratio of Oil:acetone	ARA (g/kg)		Yield
	Oil : top layer (residual oil)	Oil: bottom layer (precipitate)	(% of oil recovered from top layer)
Original oil	341		-
1:3	374	265	59
1:4	382	224	72
1:5	383	204	76
1:6	371	191	77
1:7	372	185	77
1:8	368	183	77
1:9	368	178	78

Comparative Example 5

[0045] Arachidonic oil, from the same source as used in Example 1, was placed in a freezer held at a temperature of from -18°C to-25°C for 20 hours (i.e. with no precipitate inducer added). The oil was cooled to below -20°C. Surprisingly, even at -20°C, with no inducer, no precipitate formed.

Claims

1. A process for treating a microbial oil or an oil comprising an Ω3 or Ω6 polyunsaturated fatty acid (PUFA), the process comprising:

   (a) adding a precipitate inducer to the oil;
   (b) cooling until at least a part of the oil solidifies ; and
   (c) removing the solidified matter (precipitate).

2. A process according to claim 1 wherein the oil comprises a C18, C20, C22, Ω6 or Ω3 PUFA and/or ARA, DHA,

EPA or DGLA.

3. A process according to claim 1 or 2 wherein the oil is enriched in a PUFA and/or saturated triglycerides are removed from the oil.

4. A process according to any preceding claim wherein the cooling results in the formation of two layers, a top layer having a greater concentration of PUFA than the original oil and the bottom layer (solidified matter) having a concentration of the PUFA less than the original oil.

5. A process according to any preceding claim when the solidified matter is a precipitate or sediment comprising crystals and/or the precipitate is removed either by centrifugation or filtration.

6. A process according to any preceding claim wherein the inducer facilitates formation of the precipitates by increasing the crystallisation temperature of the oil and/or is a solvent for the PUFA.

7. A process according to any preceding claim wherein the inducer has a melting point of at least -70°C and/or comprises acetone or hexane.

8. A process according to any preceding claim wherein the inducer is removed after the precipitate has been removed.

9. A process according to any preceding claim wherein the ratio of oil:inducer is from 1:1 to 1:10 and/or removal of the solidified matter takes place at a temperature below 0°C.

10. A process according to any preceding claim wherein steps (b) and (c) are performed at least twice and/or the solidifying starts at -5°C.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 31 1283

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	YOKOCHI T ET AL: "INCREASE IN THE Y-LINOLENIC ACID CONTENT BY SOLVENT WINTERIZATION OF FUNGAL OIL EXTRACTED FROM MORTIERELLA GENUS" JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY,US,AMERICAN OIL CHEMISTS' SOCIETY. CHAMPAIGN, vol. 67, no. 11, 1 November 1990 (1990-11-01), pages 846-851, XP000200851 ISSN: 0003-021X * the whole document * ---	1-10	C11B7/00
X	DATABASE WPI Section Ch, Week 199213 Derwent Publications Ltd., London, GB; Class D13, AN 1992-102413 XP002166888 & JP 04 046998 A (NISSHIN OIL MILLS LTD), 17 February 1992 (1992-02-17) * abstract * ---	1-10	
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; Database accession no. PREV198783023173, LEE K-H ET AL.: "Utilization of polyunsaturated lipids in red muscled fishes 2. Concentration refining and storage stability of polyunsaturated lipids of sardine oil" XP002166887 * abstract * & BULLETIN OF THE KOREAN FISHERIES SOCIETY, vol. 19, no. 5, 1986, pages 436-445, --- -/--	1-10	C11B

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	9 May 2001	Dekeirel, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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A	M.A. GROMPONE: "Enrichment of omega-3 PUFAs from fur seal oil" FETT WISSENSCHAFT TECHNOLOGIE- FAT SCIENCE TECHNOLOGY., vol. 94, no. 10, 1992, pages 388-394, XP002166886 CONRADIN INDUSTRIEVERLAG. LEINFELDEN ECHTERDINGEN., DE ISSN: 0931-5985 * page 390, column 1, paragraph 5 * * page 390, column 1; table 1 * * page 393; table 4 *	1-10	

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 31 1283

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09-05-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 4046998 A	17-02-1992	NONE	

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82