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(54) **STEROL EXTRACTION WITH A POLAR SOLVENT TO GIVE LOW STEROL MICROBIAL OIL**

VERFAHREN ZUM EXTRAHIEREN VON STEROL MIT EINEM POLAREN LÖSUNGSMITTEL ZUR HERSTELLUNG EINES MIKROBIELLEN ÖLES MIT NIEDRIGEM STEROLGEHALT

EXTRACTION DE STEROL A L'AIDE D'UN SOLVANT POLAIRE POUR LA PRODUCTION D'UNE HUILE MICROBIENNE A FAIBLE TENEUR EN STEROL

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(73) Proprietor: **DSM N.V.**
6411 TE Heerlen (NL)

(72) Inventors:
- **BIJL, Hendrik, Louis**
  NL-3131 ZD Vlaardingen (NL)
- **WOLF, Johannes, Hendrik**
  NL-2613 XX Delft (NL)
- **SCHAAP, Albert**
  NL-2993 BG Barendrecht (NL)

(74) Representative: **Wright, Simon Mark**
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

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- **PATENT ABSTRACTS OF JAPAN vol. 011, no. 262 (C-442), 25 August 1987 & JP 62 065689 A (AGENCY OF IND SCIENCE & TECHNOL;OTHERS: 01), 24 March 1987,**
- **JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, vol. 67, no. 11, 1 November 1990, pages 846-851, XP000200851 YOKOCHI T ET AL: "INCREASE IN THE Y-LINOLENIC ACID CONTENT BY SOLVENT WINTERIZATION OF FUNGAL OIL EXTRACTED FROM MORTIERELLA GENUS"**
- **DATABASE WPI Section Ch, Week 8737 Derwent Publications Ltd., London, GB; Class D23, AN 87-260181 XP002039741 & JP 62 179 598 A (NIPPON OILS & FATS CO LTD) , 6 August 1987**

EP 0 904 339 B9

Description

Field of the invention

[0001] The present invention relates to purified (such as by extraction) polyunsaturated fatty acid(PUFA)-containing (microbial) oils, especially oils with a sterol content of less than 1.5%.

Background of the invention

[0002] There is a growing tendency to include lipid products containing polyunsaturated fatty acids derived from various fermentation processes in foodstuffs. This is of importance in the recently established desirability to incorporate certain polyunsaturated fatty acids in an infant formula.

[0003] Various processes have been described for the fermentative production of lipids or oils containing polyunsaturated fatty acids. Examples are EP-A-155,420 for the production of γ-linolenic acid(GLA)-containing lipid from *Mortierella,* EP-A-223,960, EP-A-276,541 and WO-A-92/13086 for the production of arachidonic acid(ARA)-containing oil from *Mortierella* and/or *Pythium*, WO-A-91/07498 and WO-A-91/11918 for the production of docosahexaenoic acid (DHA)-containing oil from *Crypthecodinium cohnii* or *Thraustochytrium*, and WO-A-91/14427 for the production of eicosapentaenoic acid(EPA)-containing oil from *Nitzschia.* Typically, the microbial species producing the lipid containing the desired polyunsaturated fatty acid(s) is cultured in a suitable medium and the biomass is harvested before the desired lipid obtained.

[0004] To obtain a lipid concentrate which has a relatively high triglyceride content typically a nonpolar solvent for the lipid (e.g. hexane) or supercritical CO_2 is used in the extraction process. For example, EP-A-246,324 describes a fractional extraction process for the isolation of lipids from *Mortierella,* to obtain different extracts which are enriched in either polar or nonpolar (neutral) lipids. The neutral lipid extract still has, however, a relatively low triglyceride content (89.3%) and a high sterol content (9.4%). US patent no. 4,857,329 describes an extraction process comprising the use of supercritical CO_2 to selectively elute neutral lipids from *Mortierella* biomass. However, the triglyceride content of the lipid extract does not exceed 86%.

[0005] Yamada *et al,* Industrial applications of single cell oils, Eds. Kyle and Ratledge, 118-138 (1992) describe an arachidonic acid-containing oil extracted from *Mortierella alpina* biomass using hexane. The purified oil has a triglyceride content of 90%.

[0006] JP-A-62/179598 describes a process wherein fat (or oil)-containing material is dispersed in aqueous ethanol and crushed. The crushed material is separated from the dispersant. The separated material is extracted with n-hexane to obtain fat (or oil).

[0007] JP-A-62/065689 relates to a refined glyceride oil from which a gum such as phospholipid, triglyceride, free fatty acid has been removed by extracting a crude glyceride oil composition from a microorganism, diluting the composition with an organic solvent and contacting with a semi-permeable membrane.

[0008] Thus, until now it has not been possible to obtain a microbial triglyceride oil with a high triglyceride content, i.e. 95% or higher, using previous fermentation and extraction technology. It has also not been possible to prepare oils having a particularly low (e.g. less than 1.5%) sterol content.

Description of the invention

[0009] The present invention generally relates to a process for preparing a (microbial) oil with a high triglyceride content and a low content of "unsaponifiables", where an oil extracted, obtained or derived from a microbial biomass is treated with a polar solvent.

[0010] The present invention can thus provide a microbial (or microbially derived) oil having a high triglyceride content, such as ≥ 95%. However the oil may have a triglyceride content of at least 97%, preferably ≥ 98%, and optimally ≥ 99%. The (microbial) oil also has a low (≤ 1.5%) sterol content. Preferably the sterol content is ≤ 1%, such as ≤ 0.6%, optimally ≤ 0.3%.

[0011] The oil of the invention can be used in various compositions such as pharmaceutical (or therapeutic), cosmetic, feedstuff or food compositions (for human or animal consumption), especially in an infant formula or nutritional supplement.

[0012] A first aspect of the present invention therefore relates to a process of treating a microbially derived oil (an oil derived from a microorganism), the process comprising: (a) contacting the oil with a polar solvent to extract at least one sterol that is soluble in the solvent; and (b) separating at least some of the solvent containing the sterol from the (so treated) oil, so that the resulting oil has a sterol content of less than 1.5%.

[0013] The microbially derived oil can be extracted, obtained, or produced by one or more microorganism(s). Often this will be the same species of microorganism, but a mixture of two or more different microorganisms are envisaged

by the invention. The process of the invention may therefore be subsequent to the production of the oil itself. The oil may be one that is produced by, or exists inside (e.g. intracellularly) the microorganism(s). Alternatively, it may be obtained from a (usually aqueous) composition obtained or resulting from fermentation (of the microorganisms). This (aqueous) composition may contain the microorganisms themselves: in that case, it is usually a fermentation broth. The microorganisms (or biomass as referred to in the art) can be removed (after fermentation) by a number of methods, for example filtration, centrifugation or decantation. The oil can be extracted or obtained from this biomass.

[0014] It is usual that the microbial oil will have been obtained by extraction. This preferably will have involved extraction using a non-polar, or preferably a water-immiscible, solvent, or at least a solvent that is capable of extracting oily components. Such a solvent may be a C_{6-10} alkane, for example hexane, or (supercritical) carbon dioxide.

[0015] Different microorganisms will produce different oils. These can differ in the amount of polyunsaturated fatty acids (PUFAs) as well as in other components, and indeed the PUFAs may be in different forms, for example diglycerides, triglycerides and/or phosolipids. As such, even microbially derived oils can differ significantly from oils containing one or more of these PUFAs that have been obtained from other (e.g. animal or fish or vegetable) sources.

[0016] The microorganisms contemplated can vary widely, although preferably they will be able to produce one or more PUFAs, for example on fermentation. Microorganisms can be bacteria, algae, fungi or yeasts. Suitable fermentation processes, microorganisms and PUFA-containing oils are described in co-pending International application no. PCT/EP97/01448 (filed on 21 March 1997 in the name of Gist-brocades B.V.).

[0017] Preferred algae are of the genus *Crypthecodinium, Porphyridium* or *Nitzschia.* Preferred fungi are of the genus *Thraustochytrium, Mortierella*, *Pythium*, *Mucorales* or *Entomophthora*, in particular of the species *Mortierella alpina.*

[0018] The extracted sterol can be an alicyclic alcohol having a four conjugated ring backbone, three aromatic C_6 rings and one cyclopentane ring (e.g. desmosterol, cholesterol), an aliphatic or terpenic alcohol, tocopherol). A wax or antifoaming agent, such as polypropylene glycol may be present in the fermentation medium.

[0019] Preferred sterols include desmosterol, such as 5-desmosterol. If more than one sterol is present, then suitably 70 to 90%, e.g. 80 to 85%, of the sterols is desmosterol (e.g. for oil produced by *Mortierella*).

[0020] The oil will preferably contain at least one PUFA. This PUFA will usually have been produced by the microbe or microorganism.

[0021] PUFAs contemplated by the invention are C20 and C22 ω-3 and C18, C20 and C22 ω-6 polyunsaturated fatty acids. In particular they can include γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA is produced by algae or fungi, such as a dinoflagellate algae, for example of the genus *Crypthecodinium,* or a fungus, for example of the genus *Thraustochytrium*. GLA, DLA or ARA can be produced by fungi, such as of the genus *Mortierella, Pythium* or *Entomophthora.* EPA can be produced by an algae, such as of the genus *Porphyridium* or *Nitzschia.* Typically the oil will dominantly or only contain one PUFA, although oils can contain one or more PUFAs, for example in a lesser amount.

[0022] In the processes of the invention after the solvent has been added to the oil, the two phases (oil and solvent) will usually separate. This can easily then allow removal of one phase from the other. That can then give an oil with a low sterol content of no more than 1.5%.

[0023] A second aspect of the invention therefore relates to an oil treated or prepared by a process according to the first aspect.

[0024] A third aspect relates to a microbial oil comprising at least one polyunsaturated fatty acid (PuFA) having a sterol content of no more than 1.5%. The (total) sterol content may in fact be no more than 1%, for example less than 0.6%. By using the processes of the invention, a sterol content of no more than 0.3% can be achieved.

[0025] It will be realised that the oil of the third aspect can be prepared by using the process of the first aspect.

[0026] The different oils of the invention can be prepared, for example, by using different solvents, at different temperatures, as will be described later.

[0027] The present invention therefore provides a process for preparing an (e.g. microbial) oil, where the oil is treated with one or more polar solvents. These solvent(s) can therefore remove one or more sterols that are soluble in the solvent. This may result in concentrating or enriching of the oil. Therefore, if the oil contains triglycerides, one can concentrate or increase the triglyceride content of the oil. This may be to at least 97%, for example at least 98%, and ultimately at least 99%.

[0028] Simultaneously with increasing the triglyceride content, the solvent treatment can advantageously result in the removal of one or more impurities from the oil. In particular, this treatment can result in the lowering of the amount of "unsaponifiables". These unsaponifiables that can be removed by the solvent treatment can include the sterols, aliphatic and terpenic alcohols, waxes and antifoaming agents described earlier. Usually, the treatment of the solvent will not alter the PUFA profile or the oil so treated.

[0029] The polar solvent preferably comprises a C_{1-6} alkanol, for example ethanol. The solvent, however, may be an aqueous one. Preferred solvents therefore comprise an alcohol (e.g. ethanol) and water. However, the solvent may comprise other liquids, and these can be acetone and/or isopropanol.

[0030] If the solvent comprises ethanol, this may have a water content of from 0 to 20%, such as from 1 to 7%, and

optionally from 2 to 4%. If the solvent comprises methanol, acetone and/or isopropanol (IPA), then the water content is preferably 0 to 2%, 5 to 50% and 5 to 15%, respectively. The solvent may therefore comprise a mixture of two or more liquids. It has been found that ethanol containing a small amount of water (e.g. 97% ethanol, 3% water) can significantly improve the yield of triglyceride after solvent treatment. This is because triglycerides are relatively insoluble in this particular solvent. Having the solvent at a temperature of from 15 to 30°C, e.g. 20 to 25°C, also reduces the amount of triglycerides that dissolved in the ***[deletion(s)]*** solvent.

[0031] By using different solvents one can vary the amount of sterol (or indeed PUFA) that is extracted. As has been discussed above, a mix of ethanol and water can provide a high yield of triglycerides since although this solvent will dissolve sterols, triglycerides are nevertheless relatively insoluble in it.

[0032] The PUFA will generally exist in several forms, such as triglycerides and diglycerides. These compounds are effectively a glycerol molecule with one or more (although usually only one) of the PUFAs attached to this backbone. Preferably the triglyceride form will be dominant. In the oil of the third aspect (e.g. from the process of the first aspect), the amount of diglycerides present is preferably no more than 2.2%, and preferably less than 1%. The solvent used here is preferably at a temperature of from 10 to 40°C, e.g. 20 to 30°C.

[0033] The amount of sterol to be extracted, or the triglyceride content, can be adjusted by varying several process parameters. For example, one can adjust the ratio of solvent to oil, the temperature during extraction and/or by repeating the extraction process. If more than one extraction is to be performed, a counter-current extraction process is preferred, which can minimise triglyceride losses.

[0034] Usually the oil will be a crude oil obtained after extraction from a (e.g. dried) microbial biomass with a suitable solvent, followed by evaporation of that (water immiscible) solvent. The oil may be subjected to-one or more refining steps prior to the process of the invention.

[0035] The oil of the invention, or one which results from a process of the first aspect, can be used for various purposes without further processing, or can be additionally subjected to one or more refining steps. The oil can be used as an additive or a supplement, for example in food compositions, such as an infant formula. It may however also be used in cosmetic or pharmaceutical compositions. The invention in a further aspect therefore relates to a composition, such as a food stuff, feed or pharmaceutical composition or a cosmetic composition, which comprises, or to which has been added, an oil of the invention. Preferred compositions are foods, such as infant formula or a nutritional supplement.

[0036] The oil of the invention can therefore have a low sterol and optionally alow diglyceride content. Optionally, it may also have a high triglyceride content. This makes the oil particularly suitable for nutritional purposes, and can be used as 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 in foods, feeds or foodstuffs, suitable for human or animal consumption. Suitable examples are health drinks and bread. Particularly contemplated is the use in infant formula, or in cosmetics.

[0037] Preferred features and characteristics of one aspect of the invention are equally applicable to another aspect *mutatis mutandis.*

[0038] The invention will now be described, by way of example, with reference to the following Examples which are provided merely for means of illustration, and are not to be construed as being limiting on the invention.

COMPARATIVE EXAMPLE 1

Recovery of crude ARA oil from *M. alpina* biomass

[0039] 500 1 of broth obtained after *Mortierella alpina* fermentation was filtered in a membrane filter press (cloth type: propex 46K2). The broth was filtered with a pressure difference of 0.2 bar. Within 21 minutes 500 1 broth was filtered over a total filter area of 6.3 m^2 which resulted in an average flow of about 230 l/m^2h. The filter cake was washed in 30 minutes with 10 cake volumes of tap water at an average flow rate of 320 l/m^2h.

[0040] The cake was squeezed at 5.5 bar for 30 minutes which resulted in a dry matter content of the recovered biomass of about 45%.

[0041] Extrusion was performed on the resulting biomass cake using a single screw extruder with a profiled barrel and a universal screw. The dieplate used for extrusion had holes of diameter 2mm.

[0042] Drying of the extrudate was performed in a fluidized bed dryer with air (8000 Nm^3/m^2h). The setpoint of the bed temperature was 80°C. The diameter of the dried extruded biomass was 2mm and its dry matter content after drying was about 96%.

[0043] A crude arachidonic acid-containing oil (ARA oil) was then extracted from the extrudate using hexane as a solvent.

EXAMPLES 2 AND 3

Treatment of microbial ARA oil with 100% ethanol

[0044] 5ml of crude ARA oil was extracted from the extrudate of Example 1 with a volume of 100% ethanol for 1 minute by hand-shaking. Subsequently, the bottom and toplayers were separated by centrifugation for 5 minutes at 5000 rpm. The samples were analyzed by means of (600 Mhz) NMR (for tri- and di-glycerides, sterols (only desmosterol content measured) and antifoaming agent).

[0045] Extraction of crude ARA oil with 9 volumes of 100% ethanol at two different temperatures resulted in an oil with a decreased level of sterol and diglyceride (DG) and in an increased level of triglyceride (TG, see Table 1). The yield of TG is the percentage of triglyceride remaining in the oil after solvent extraction. Also antifoaming agent was removed and found in the ethanol after extraction. However, the yield of triglycerides was low due to the fact that some of the TG dissolved (and was thus removed in) the ethanol.

Table 1

Extraction of crude ARA oil with 100% ethanol (data for treated oil)						
Ex	solvent	temp.	% TG	% DG	% sterol	yield TG
-	Control	---	96.2	2.2	1.6	100
2	EtOH 100%	ambient	98.2	0.7	1.1	73.8
3	EtOH 100%	60°C	98.5	0.7	0.8	43.2

Key:
TG: triglycerides
DG: diglycerides
Sterol: as desmosterol

EXAMPLES 4 TO 9

Treatment of microbial ARA oil with 97% ethanol

[0046] Examples 2 and 3 were repeated except using 97% ethanol at varying volumes relative to the oil.

[0047] Extraction of crude ARA oil with 1, 3 and 9 volumes of 97% ethanol resulted in an oil with a decreased level of sterol and diglyceride and in an increased level of triglyceride (see Table 2).

[0048] The yield of triglycerides was above 92% due to the fact that not much oil dissolves in 97% ethanol. At ambient temperature (about 20°C), a higher yield of triglycerides and a better removal of diglycerides and sterols was observed. Remarkably no ethanol was found in the treated oil.

Table 2

Extraction of crude ARA oil with 97% ethanol (data for treated oil)							
Ex	solvent	temp.	vol EtOH	% TG	% DG	% sterol	yield TG
-	Control	---	0	96.2	2.2	1.6	100
4	EtOH 97%	ambient	1	96.7	1.8	1.4	92.9
5	EtOH 97%	ambient	3	97.8	1.1	1.1	95.0
6	EtOH 97%	ambient	9	98.9	0.4	0.7	96.2
7	EtOH 97%	60°C	1	96.4	2.0	1.6	99.7*
8	EtOH 97%	60°C	3	97.7	1.1	1.2	92.4
9	EtOH 97%	60°C	9	98.3	0.6	1.1	93.7

Key:
TG: triglycerides
DG: diglycerides
Sterol: as desmosterol
* Due to the increase of the lower (oil) phase because the ethanol partly dissolved into the oil and so phase separation was more difficult.

[0049] The ethanol phase was also analyzed after extraction and a significant increase in sterols was observed. Also the antifoam agent (polypropylene glycol) was extracted and found in the ethanol phase (see Table 3).

Table 3

Extraction of crude ARA oil with 97% ethanol (data for ethanol phase)							
Ex	solvent	temp.	vol EtOH	% TG	% DG	% antifoam	% sterol
4	EtOH 97%	ambient	1	60.9	20.8	4.1	14.2
5	EtOH 97%	ambient	3	73.1	15.3	1.3	10.2
6	EtOH 97%	ambient	9	83.0	10.0	0.7	6.3
7	EtOH 97%	60°C	1	66.1	18.3	3.7	11.9
8	EtOH 97%	60°C	3	78.6	12.5	1.1	7.8
9	EtOH 97%	60°C	9	87.9	7.1	0.4	4.5

Key:
TG: triglycerides
DG: diglycerides
Sterol: as desmosterol

Claims

1. A process of treating an oil derived from a microorganism, the process comprising:

   (a) contacting the oil with a polar solvent to extract at least one sterol that is soluble in the solvent; and
   (b) separating at least some of the solvent containing the sterol from the oil, so that the resulting oil has a sterol content of less than 1.5%.

2. A process according to claim 1 when the oil is obtained or extracted from a composition resulting from a fermentation, optionally a fermentation broth.

3. A process according to claim 2, wherein the oil is derived, obtained or extracted from microorganisms present in the composition.

4. A process according to claim 2 wherein the microorganisms are first removed from the composition, optionally by filtering the composition.

5. A process according to any one of claims 2 to 4 wherein the microorganisms are dried before the oil is obtained.

6. A process according to any one of claims 2 to 5 wherein the oil has been extracted using a solvent for triglycerides.

7. A process according to claim 6 when the solvent is hexane, supercritical carbon dioxide or isopropanol.

8. A process according to any preceding claim wherein the oil is produced by, or the microorganism is, a bacteria, fungus, yeast or algae.

9. A process according to claim 8 when the microorganism is of the genus *Crypthecodinium, Mucorales, Thraustochytrium, Mortierella, Pythium*, *Entomophthora, Porphyridium* or *Nitzschia*.

10. A process according to any preceding claim wherein the oil is derived from *Mortierella alpina* or wherein the sterol is produced by, or is present intracellularly inside, the microorganism.

11. A process according to any one of the preceding claims wherein the sterol is desmosterol.

12. A process according to any one of the preceding claims when the oil comprises at least one polyunsaturated fatty acid (PUFA).

13. A process according to claim 12 wherein the PUFA is a C18, C20 or C22 ω-3 or ω-6 polyunsaturated fatty acid.

14. A process according to claim 13 wherein the PUFA is GLA, DLA, ARA, EPA or DHA.

15. A process according to claim any one of the preceding claims wherein the polar solvent comprises a C_{1-6} alkanol or acetone.

16. A process according to any one of claims 13 to 15 wherein the solvent is ethanol or isopropanol or ethanol and from 1 to 5% water.

17. A process according to any one of the preceding claims wherein the amount of solvent used in the extraction is from 1 to 9 times the volume of the oil to be treated.

18. An oil treated or prepared by a process according to any one of the preceding claims.

19. A microbial oil comprising at least one polyunsaturated fatty acid (PUFA) and having a sterol content of no more than 1.5%.

20. An oil according to claim 18 or 19 having a sterol content of no more than 1%.

21. The use of an oil according to any one of claims 18 to 20 in a pharmaceutical, cosmetic, feed or foodstuff (for consumption by humans or animals) composition.

22. A composition comprising, or to which has been added, an oil according to any one of claims 18 to 20.

23. A composition according to claim 22 which is a foodstuff, feed, or pharmaceutical composition or a nutritional supplement for consumption by humans or animals.

24. A composition according to claim 22 or 23 which is an infant formula or a cosmetic composition.

Patentansprüche

1. Verfahren zur Behandlung eines Öls, erhalten aus einem Mikroorganismus, das Verfahren umfassend:

(a) Inkontaktbringen des Öls mit einem polaren Lösungsmittel, um zumindest ein Sterol zu extrahieren, das in dem Lösungsmittel löslich ist; und
(b) Abtrennen zumindest eines Teiles des Lösungsmittels, das das Sterol aus dem Öl enthält, so dass das resultierende Öl einen Sterolgehalt von weniger als 1,5 % aufweist.

2. Verfahren nach Anspruch 1, wenn das Öl erhalten oder extrahiert wird aus einer Zusammensetzung, die aus einer Fermentierung, gegebenenfalls einer Fermentationsbrühe resultiert.

3. Verfahren nach Anspruch 2, in dem das Öl gewonnen, erhalten oder extrahiert wird aus Mikroorganismen, die in der Zusammensetzung vorliegen.

4. Verfahren nach Anspruch 2, in dem die Mikroorganismen zuerst aus der Zusammensetzung entfernt werden, gegebenenfalls durch Filtrieren der Zusammensetzungen.

5. Verfahren nach einem der Ansprüche 2 bis 4, in dem die Mikroorganismen getrocknet werden, bevor das Öl erhalten wird.

6. Verfahren nach einem der Ansprüche 2 bis 5, in dem das Öl unter Verwendung eines Lösungsmittels für Triglyceride extrahiert wurde.

7. Verfahren nach Anspruch 6, wenn das Lösungsmittel Hexan, überkritisches Kohlendioxid oder Isopropanol ist.

8. Verfahren nach einem der vorstehenden Ansprüche, in dem das Öl durch Bakterien, Pilz, Hefe oder Algen erzeugt

wird oder der Mikroorganismus Bakterien, Pilz, Hefe oder Algen darstellt.

9. Verfahren nach Anspruch 8, wenn der Mikroorganismus zur Gattung *Crypthecodinium, Macorales, Thraustochytrium, Mortierella, Pythium, Entomophthora, Porphyridium* oder *Nitzschia* gehört.

10. Verfahren nach einem der vorstehenden Ansprüche, in dem das Öl von *Mortierella alpina* erhalten wird oder in dem das Sterol durch den Mikroorganismus erzeugt wird oder in diesem intrazellulär vorliegt.

11. Verfahren nach einem der vorstehenden Ansprüche, in dem das Sterol Desmosterol ist.

12. Verfahren nach einem der vorstehenden Ansprüche, wenn das Öl zumindest eine mehrfach ungesättigte Fettsäure (PUFA) umfasst.

13. Verfahren nach Anspruch 12, in dem die PUFA eine mehrfach ungestättigte C18-, C20- oder C22-ω-3- oder -ω-6-Fettsäure ist.

14. Verfahren nach Anspruch 13, in dem die PUFA GLA, DLA, ARA, EPA oder DHA ist.

15. Verfahren nach einem der vorstehenden Ansprüche, in dem das polare Lösungsmittel ein C_1-C_6-Alkanol oder Aceton umfasst.

16. Verfahren nach einem der Ansprüche 13 bis 15, in dem das Lösungsmittel Ethanol oder Isopropanol oder Ethanol und 1 bis 5 % Wasser ist.

17. Verfahren nach einem der vorstehenden Ansprüche, in dem die Menge des in der Extraktion verwendeten Lösungsmittels das 1- bis 9-fache des Volumens des zu behandelnden Öls darstellt.

18. Öl, behandelt oder hergestellt durch ein Verfahren nach einem der vorstehenden Ansprüche.

19. Mikrobielles Öl, umfassend zumindest eine mehrfach ungesättigte Fettsäure (PUFA) und einen Sterolgehalt von nicht mehr als 1,5 % aufweisend.

20. Öl nach Anspruch 18 oder 19, einen Sterolgehalt von nicht mehr als 1 % aufweisend.

21. Verwendung eines Öls nach einem der Ansprüche 18 bis 20 in einer pharmazeutischen, kosmetischen, Futtermittel- oder Lebensmittelzubereitung (für den Verbrauch durch Menschen oder Tiere).

22. Zubereitung, umfassend ein Öl nach einem der Ansprüche 18 bis 20, oder der ein Öl nach einem der Ansprüche 18 bis 20 zugegeben wurde.

23. Zubereitung nach Anspruch 22, die eine Lebensmittel-, Futtermittel- oder pharmazeutische Zubereitung oder ein Nahrungsergänzungsmittel für den Verbrauch durch Menschen oder Tiere darstellt.

24. Zubereitung nach Anspruch 22 oder 23, die eine Kindernahrung oder eine kosmetische Zubereitung darstellt.

Revendications

1. Procédé de traitement d'une huile dérivée d'un microorganisme, lequel procédé comprend

(a) la mise en contact de l'huile avec un solvant polaire afin d'extraire au moins un stérol soluble dans le solvant, et
(b) séparation d'au moins une partie du solvant contenant le stérol de l'huile de manière à ce que l'huile obtenue ait une teneur en stérol inférieure à 1,5 %.

2. Procédé selon la revendication 1, dans lequel l'huile est obtenue ou extraite d'une composition provenant d'une fermentation, éventuellement d'un bouillon de fermentation.

3. Procédé selon la revendication 2, dans lequel l'huile est dérivée, obtenue ou extraite de microorganismes présents dans la composition.

4. Procédé selon la revendication 2, dans lequel les microorganismes sont d'abord séparés de la composition, éventuellement par filtration de la composition.

5. Procédé selon l'une quelconque des revendications 2 à 4, dans lequel les microorganismes sont séchés avant l'obtention de l'huile.

6. Procédé selon l'une quelconque des revendications 2 à 5, dans lequel l'huile a été extraite à l'aide d'un solvant capable de dissoudre les triglycérides.

7. Procédé selon la revendication 6, dans lequel le solvant est l'hexane, le dioxyde de carbone supercritique ou l'isopropanol.

8. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'huile est produite par une bactérie, un champignon, une levure ou une algue ou le microorganisme est une bactérie, un champignon, une levure ou une algue.

9. Procédé selon la revendication 8, dans lequel le microorganisme est choisi parmi les genres *Crypthecodinium, Mucorales*, *Thraustochytrium, Mortierella*, *Pythium*, *Entomophthora, Porphyridium ou Nizschia.*

10. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'huile est dérivée de *Mortierella alpina* ou dans lequel le stérol est produit par ou est présente dans les cellules des microorganismes.

11. Procédé selon l'une quelconque des revendications précédentes, dans lequel le stérol est le desmostérol.

12. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'huile comprend au moins un acide gras polyinsaturé (AGPI).

13. Procédé selon la revendication 12, dans lequel l'AGPI est un acide gras en C_{18}, C_{20} ou C_{22} polyinsaturé en ω-3 ou ω-6.

14. Procédé selon la revendication 13, dans lequel l'AGPI est choisi parmi GLA, DLA, ARA, EPA et DHA.

15. Procédé selon l'une quelconque des revendications précédentes, dans lequel le solvant polaire comprend un alcanol en C_{1-6} ou de l'acétone.

16. Procédé selon l'une quelconque des revendications 13 à 15, dans lequel le solvant est l'éthanol ou l'isopropanol ou l'éthanol contenant de 1 à 5 % d'eau.

17. Procédé selon l'une quelconque des revendications précédentes, dans lequel la quantité de solvant utilisé pour l'extraction est comprise entre 1 et 9 fois le volume d'huile à traiter.

18. Huile traitée ou préparée par un procédé selon l'une quelconque des revendications précédentes.

19. Huile d'origine microbienne comprenant au moins un acide gras polyinsaturé et ayant une teneur en stérol n'excédant pas 1,5 %.

20. Huile selon la revendication 18 ou 19 ayant une teneur en stérol n'excédant pas 1 %.

21. Utilisation d'une huile selon l'une quelconque des revendications 18 à 20 dans un produit pharmaceutique, un produit cosmétique ou dans un aliment (pour humains ou animaux)

22. Composition comprenant une huile selon l'une quelconque des revendications 18 à 20, ou à laquelle on a ajoutée une telle huile.

23. Composition selon la revendication 22, qui est un aliment, de la nourriture pour animaux ou une composition

pharmaceutique ou un supplément nutritionnel destiné à être consommé par des humains ou des animaux.

24. Composition selon la revendication 22 ou 23, qui est une préparation pour nourrisson ou une composition cosmétique.