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(54) Benævnelse: **FREMGANGSMÅDE TIL ISOLERING AF LIPIDER FRA EN LIPID-INDEHOLDENDE BIOMASSE VED HJÆLP AF HYDROFOB SILICA**

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DESCRIPTION

Description

[0001] The current invention relates to a method of isolating lipids from a lipids containing biomass with aid of hydrophobic silica.

[0002] PUFAs (polyunsaturated fatty acids) containing lipids are of high interest in the feed, food and pharmaceutical industry. Due to overfishing there is a high need for alternative sources for PUFAs containing lipids besides fish oil. It turned out that besides certain yeast and algal strains in particular microalgal cells like those of the order Thraustochytriales are a very good source for PUFAs containing lipids.

[0003] But with respect to microbial organisms and in particular cells of the order Thraustochytriales, which produce the PUFAs containing lipids, the isolation of the oil from the cells turned out as a particular problem. The most effective way of isolating the oil was the use of organic solvents like hexane. But the use of organic solvents leads to hazardous operating conditions, requires the use of expensive explosion-proof equipment and requires the implementation of an expensive solvent recovery process to avoid pollution of the environment.

[0004] In the attempt to avoid the use of organic solvents, as an effective alternative way for isolating the oil has turned out the salting-out of the oil with high amounts of sodium chloride. But the use of high amounts of sodium chloride leads to a delipidated biomass by-product which due to the high salt content cannot be utilized as a feed ingredient, so that the process is not very sustainable. Further, the high salt concentration leads to fast corrosion of the used steel equipment.

[0005] Thus, it was the object of the current invention to provide an effective method for isolating a lipid, in particular a PUFAs containing lipid, from lipids containing cells, in particular of the order Thraustochytriales, and simultaneously avoiding not only the need of organic solvents, but further avoiding the need of high amounts of salts and preferably also avoiding the use of high amounts of base and/or a high pH for realizing the effective isolation of the oil from the cells.

[0006] It was a further object of the current invention to provide a method for isolating a lipid, in particular a PUFAs containing lipid, from lipids containing cells, in particular of the order Thraustochytriales, and simultaneously to provide a delipidated biomass which can be utilized in a commercial way, preferably in the agricultural field.

[0007] It turned out that an effective isolation of the lipid from the biomass can be realized, if the biomass, in particular after lysing, is incubated with hydrophobic silica, preferably at about

neutral pH and at a temperature of between 50°C and 100°C. By keeping the temperature well below 100°C and avoiding high pH values, it was possible to prohibit at least essentially saponification of the fatty acid esters. Thus, an environmentally friendly, sustainable as well as mild method for isolating lipids from the cells of the biomass is provided.

[0008] As further there was no need to apply alkaline conditions and thus no alkaline agents and further also no organic solvents have to be added, a biomass was obtained which contained very low amounts of salts and/or is essentially free of organic solvents and by that turned out as very useful for applications in particular in the agricultural field. The present invention is defined in claims 1 to 14.

[0009] Thus, a first subject of the current invention is a method of isolating a lipid from a lipids containing biomass, comprising the following steps:

1. a) Providing a biomass suspension, wherein the cells of the biomass contain in average at least 10 wt.-% of lipids;
2. b) Optionally lysing the cells of the biomass;
3. c) Adding hydrophobic silica to the suspension;
4. d) Heating the suspension thus obtained to a temperature of at least 50°C, in particular 50 to 100°C, preferably 65 to 95°C, and incubating the suspension for at least 20 minutes, preferably 20 minutes to 30 hours, in particular 30 minutes to 26 hours, more preferably 1 hour to 24 hours, 2 to 20 hours, 3 to 15 hours or 4 to 10 hours;
5. e) Separating the oil containing light phase from the cell-debris containing aqueous phase.

[0010] Preferably the steps are carried out in the order as depicted, but optionally hydrophobic silica may be added before lysing of the suspension or, on the other hand, hydrophobic silica may be added after the suspension has already been heated.

[0011] Preferably the biomass suspension is further concentrated before or after addition of the hydrophobic silica to a total dry matter (TDM) content of 20 to 60 wt.-%, if it has a TDM content outside of this range.

[0012] Thus, the disclosure describes a method of isolating lipids from a lipids containing biomass, comprising the following steps:

1. a) Providing a suspension of a biomass comprising cells which contain a PUFAs containing lipid;
2. b) Optionally lysing the cells of the biomass;
3. c) Concentrating the suspension to a total dry matter (TDM) content of 20 to 60 wt.-%, preferably 25 to 60 wt.-%, more preferably 30 to 55 wt.-%, if the suspension has a lower TDM content;
4. d) Adding hydrophobic silica to the suspension;

5. e) Heating the suspension thus obtained to a temperature of at least 50°C, in particular 50 to 100°C, preferably 65 to 95°C, and incubating the suspension for at least 20 minutes, preferably 20 minutes to 30 hours, in particular 30 minutes to 26 hours, more preferably 1 hour to 24 hours, 2 to 20 hours, 3 to 15 hours or 4 to 10 hours;
6. f) Separating the oil set free by mechanical means from the cell-debris containing aqueous phase.

[0013] In this embodiment steps (a) to (f) are preferably carried out in the order as depicted. But optionally concentration of the biomass may also be carried out before lysing of the cells or hydrophobic silica may be added before lysing and/or concentrating of the suspension or, on the other hand, hydrophobic silica may be added after the suspension has already been heated.

[0014] The steps of lysing the cells, concentration of the suspension, addition of hydrophobic silica and incubation of the suspension with hydrophobic silica are preferably carried out under mechanical mixing, preferably by stirring or agitating of the respective suspension. - Stirring and/or agitating may be carried out accordingly by using a stirrer and/or an agitator. In particular low shear agitation and/or axial-flow agitation may be applied, in particular as disclosed in WO 2015/095694. Impellers suitable for agitating include in particular straight blade impellers, Rushton blade impellers, axial flow impellers, radial flow impellers, concave blade disc impellers, high-efficiency impellers, propellers, paddles, turbines and combinations thereof.

[0015] In the methods according to the invention, the addition of silica leads to the breaking of the emulsion as contained in the suspension into an oil containing light phase and a water, cell debris and salts containing heavy phase. This breaking of the emulsion is also called "demulsification" in the context of this application.

[0016] In a preferred embodiment of the invention, hydrophobic silica is added to the suspension to adjust a final concentration in the suspension of between 0.005 and 0.25 wt.-% of hydrophobic silica. In particular, hydrophobic silica is added in an amount to adjust a final concentration of between 0.008 and 0.20 wt.-%, preferably 0.012 to 0.15 wt.-%, more preferably 0.015 to 0.10 wt.-%. According to the invention, "hydrophobic silica" may refer to a single kind of hydrophobic silica or to a mixture of different kinds of hydrophobic silicas.

[0017] Hydrophobic silicas are registered under CAS No. 68611-44-9 and are commercially available, for example, under the trade names Sipernat® or Aerosil® (Evonik Industries, Germany). Hydrophobic silicas are characterized by hydrophobic groups chemically bonded to the surface. The chemical groups are preferably alkyl or polydimethylsiloxane groups. Hydrophobic silica can be obtained, for example, by chemical treatment of hydrophilic silica with alkoxy silanes, silazanes or siloxanes.

[0018] Hydrophobic silicas preferably used according to the invention have a methanol wettability of at least 40%, preferably at least 45%, particularly preferably at least 50%, particularly 40 to 65%, especially 45 to 60%.

[0019] The methanol wettability is a measure of the hydrophobicity of the silica powder. To determine this value, a certain amount of silica powder is weighed into water. The silica powder remains here on the surface. The amount of methanol required for wetting the powder is then determined. "Methanol wettability" is here understood to mean the methanol content of a methanol-water mixture in % by volume in which 50% of the hydrophobic silica sediments.

[0020] The hydrophobic silicas according to the invention preferably have a tamped density (unsieved; based on ISO 787-11) of 100 to 200 g/l, preferably 125 to 175 g/l. The particle size of the hydrophobic silica (d50; laser diffraction; based on ISO 13320-1) is preferably from 2 to 50 µm, more preferably from 5 to 20 µm, in particular from 8 to 15 µm. Loss on drying of the hydrophobic silica (2 hours at 105°C; based on ISO 787-2) is preferably at most 10%, particularly preferably at most 6%. Ignition loss of the hydrophobic silica (2 hours at 1000°C; based on ISO 3262-1) is preferably at most 10%, particularly preferably at most 6%.

[0021] The silicon dioxide content of the hydrophobic silica is preferably at least 95% by weight, particularly preferably at least 97% by weight (based on ISO 3262-19). The carbon content of the hydrophobic silica is preferably at most 3.5%, in particular 0.2 to 3.5%, by weight, particularly at most 2%, in particular 0.5 to 2%, by weight (based on ISO 3262-19). The pH of the hydrophobic silica (5% in a 1:1 mixture of water and methanol; based on ISO 787-9) is preferably from 7 to 10.5, particularly preferably from 7.5 to 9.

[0022] Hydrophobic silica(s) which can be used in accordance with the invention are, for example, obtainable under the trade names Sipernat® D 10, Sipernat® D13, Sipernat® D 15, Sipernat® D 17 and Aerosil® types R, RX, RY and NX, in particular Aerosil® R 202 (Evonik Industries, Germany).

[0023] In a preferred embodiment of the invention, the hydrophobic silica is used in form of a demulsifier mixture which contains besides the hydrophobic silica at least one surfactant. The at least one surfactant may be selected from the group consisting of nonionic, anionic, zwitterionic, ampholytic and cationic surfactants. In a preferred embodiment of the invention the at least one surfactant is preferably a nonionic surfactant.

[0024] Preferred anionic surfactants are alkyl sulfates, alkyl polglycol ether sulfates and ether carboxylic acids with 10 to 18 C atoms in the alkyl group and up to 12 glycol ether groups in the molecule, sulfosuccinic acid mono and dialkyl esters with 8 to 18 C atoms in the alkyl group and sulfosuccinic acid mono-alkylpolyoxyethyl esters with 8 to 18 C atoms in the alkyl group and 1 to 6 oxyethylene groups, monoglyceride sulfates, alkyl and alkenyl ether phosphates as well as condensates of protein and fatty acids.

[0025] Zwitterionic surfactants carry at least a quaternary ammonium group and at least one -

COO<-> - or SO3<-> group in the molecule. Particularly suitable zwitterionic emulsifiers are the so-called betaines such as N-alkyl-N,N-dimethylammonium glycinates, N-acyl-aminopropyl-N,N-dimethylammonium glycinates and 2-alkyl-3-carboxymethyl-3-hydroxyethyl-imidazolines, each having 8 to 18 carbon atoms in the alkyl or acyl group, as well as cocoacylaminoethylhydroxyethylcarboxymethyl glycinate.

[0026] Exemplary nonionic surfactants usable according to the invention are linear or branched C8-C30 fatty acids and their Na, K, ammonium, Ca, Mg and Zn salts; fatty acid alkanolamides; fatty acid glucamides; N-alkylglucamides; and C8-C22 alkylamine-N-oxides.

[0027] Preferred nonionic surfactants according to the invention are alkoxylated, in particular ethoxylated and/or propoxylated, preferably ethoxylated, compounds as well as alkylated sugars and in particular alkoxylated alkylated sugars.

[0028] Examples for alkoxylated compounds are alkoxylated fatty alcohols, sugar alcohols, fatty acids, alkylphenols, castor oil, hydrogenated castor oil, fatty acid alkanolamides and fatty acid amines; in particular addition products of 4 to 30 moles ethylene oxide and/or 0 to 5 moles propylene oxide on linear C8-C22-fatty alcohols, on C12-C22-fatty acids and on C8-C15-alkylphenols; C12-C22 fatty acid mono and diesters of addition products of 1 to 30 moles ethylene oxide on C3-C6 polyols, particularly on glycerin, ethylene oxide and polyglycerin; addition products on methylglucoside fatty acid esters, addition products of 5 to 60 moles ethylene oxide on castor oil and hydrogenated castor oil, partial esters of polyols containing 3-6 carbon atoms with C8-C22 fatty acids; addition products of 1 to 30 moles ethylene oxide to fatty acid alkanolamides and fatty acid amines.

[0029] Further examples for alkoxylated compounds are alkoxylated fatty acid alkyl esters of the formula R<1>CO-(OCH2CHR<2>)xOR<3> in which R<1>CO stands for a linear or branched, saturated and/or unsaturated acyl group with 6 to 22 carbon atoms, R<2> for hydrogen or methyl, R<3> for linear or branched alkyl groups with 1 to 4 carbon atoms and x for numbers from 1 to 20.

[0030] Further examples of nonionic surfactants are alkyl mono-, oligo- and polyglycosides corresponding to the general formula RO-(Z)x wherein R stands for a C8-C22 alkyl, in particular C8-C16 alkyl, Z for sugar and x for the number of sugar units.

[0031] Any mono-, oligo- or polysaccharide can be added as the sugar building block Z. Normally, sugars having 5 or 6 carbon atoms as well as the corresponding oligosaccharides are added, for example, glucose, fructose, galactose, arabinose, ribose, xylose, lyxose, allose, altrose, mannose, gulose, idose, talose and/or sucrose. Preferred sugar building blocks are glucose, fructose and sucrose with glucose being particularly preferred. The inventively usable alkyl polyglycosides comprise an average of 1.1 to 5, preferably 1.1 to 2.0, particularly preferably 1.1 to 1.8 sugar units. The alkoxylated homologs of the cited alkyl polyglycosides can also be used according to the invention. These homologs preferably comprise on average up to 10 ethylene oxide and/or propylene oxide units per alkyl glycoside unit.

[0032] In a very preferred embodiment of the invention, fatty acid esters of sugar alcohols and/or alkoxylated derivatives of such compounds are used as nonionic surfactants. Preferred sugar alcohols in this context are glycerol, polyglycerol, sorbitol, isosorbide and sorbitans, in particular 1,4-sorbitan. Examples of such fatty acid esters of sugar alcohols are polyglyceryl-2-dipolyhydroxy stearate and polyglyceryl-3-diisostearate.

[0033] Very preferred examples of such fatty acid esters of sugar alcohols are esters of sorbitan, in particular of 1,4-sorbitan, with C12-C22 fatty acids, preferably with C16-C18 fatty acids, wherein the ester bond is preferably formed with the omega hydroxyl group of the sorbitan.

[0034] Particular examples are sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan sesquioleate, sorbitan trioleate and sorbitan isostearate, with sorbitan monooleate being particularly preferred. Sorbitan monooleate is commercially available under the tradename Span® 80 (Croda, USA).

[0035] Very preferred examples of such alkoxylated fatty acid esters of sugar alcohols are esters of ethoxylated sorbitan, in particular of ethoxylated 1,4-sorbitan, with C12-C22 fatty acids, preferably with C16-C18 fatty acids, wherein preferably all free hydroxyl groups of the sorbitan do carry 1 to 5, preferably 1 to 3, ethoxylate groups and wherein the fatty acid bond is formed with the ethoxylated omega hydroxyl group of the sorbitan. These compounds are also called polysorbates. Particular examples are PEG-20 sorbitan monolaurate, PEG-4 sorbitan monolaurate, PEG-20 sorbitan monopalmitate, PEG-20 sorbitan monostearate, PEG-4 sorbitan monostearate, PEG-20 sorbitan tristearate and PEG-20 sorbitan monooleate, with PEG-20 sorbitan monooleate being particularly preferred. PEG-20 sorbitan monooleate, also known as polysorbate 80, is commercially available under the tradename Tween® 80 (Croda, USA).

[0036] In a preferred embodiment of the invention, a mixture of at least two nonionic surfactants is used in the demulsifier composition. Preferably a mixture of a nonionic surfactant with a low HLB of preferably below 8, in particular from 3 to 6, and a nonionic surfactant with a high HLB of preferably above 12, in particular from 13 to 17, is used in the demulsifier composition. Preferred examples for this mixture is a mixture of Span® 80 and Tween® 80 (Croda, USA) or the mixture of Brij® 92 and Brij® 98 (Uniqema, USA). (Concerning the definition of the HLB see Römpp-Lexikon Chemie (Eds.: J. Falbe, M. Regitz), 10th edition, Georg Thieme Verlag Stuttgart, New York, (1997), page 1764).

[0037] Suitable emulsifiers with an HLB of below 8 are in particular compounds of the general formula R<1> -O-R<2> , in which R<1 > is a primary linear alkyl, alkenyl or acyl group having 18 to 30 carbon atoms, like the behenyl, erucyl or arachidyl group, and R<2 > is hydrogen, a group of formula -(CnH2nO)x-H with x=1 or 2 and n=2 to 4 or a polyhydroxyalkyl group with 4 to 6 carbon atoms and 2 to 5 hydroxy groups, like pentaerythreitol, trimethylol propane, diglycerin or methylglucose.

[0038] In case that the hydrophobic silica is used as a demulsifier mixture in combination with at least one surfactant, then the ratio of hydrophobic silica to surfactant preferably varies from 1:50 to 50:1. In a preferred embodiment of the invention, the hydrophobic silica is contained in the demulsifier mixture in an amount of from 1 wt.-% to 50 wt.-%, preferably in an amount of 2 to 25 wt.-%, in particular in an amount of 3 to 15 wt.-%.

[0039] Preferably the surfactant(s) form(s) the residual part of the demulsifier mixture, but besides surfactants further ingredients may be included in the demulsifier mixture, for example to modulate the physical properties of the demulsifier mixture.

[0040] In a very preferred embodiment of the invention, a demulsifier mixture is used, which comprises hydrophobic silica in an amount of 3 to 15 wt.-% and at least one, preferably two, nonionic surfactant(s) in an amount of at least 85 wt.-%, wherein the nonionic surfactant(s) is/are preferably selected from fatty acid esters of sugar alcohols and/or alkoxylated derivatives of such compounds, in particular from fatty acid esters of sorbitan, preferably sorbitan monooleate, and ethoxylated fatty acid esters of sorbitan, preferably PEG-20 sorbitan monooleate, and mixtures thereof.

[0041] In another preferred embodiment of the invention the hydrophobic silica is used in combination with an oil, in particular in combination with a triglyceride containing oil, such as microalgal oil or vegetable oil, wherein examples for vegetable oils are soybean oil, canola oil and corn oil. If the hydrophobic silica is used in combination with an oil, then the ratio of hydrophobic silica to oil preferably varies from 1:50 to 50:1, in particular from 1:1 to 1:20.

[0042] In a particular embodiment of the invention, the hydrophobic silica is employed in combination with at least one nonionic surfactant and simultaneously in combination with at least one oil, in particular in combination with at least one triglyceride containing oil.

[0043] As according to the invention, it is not necessary to raise or lower the pH value, the reaction is preferably carried out at about neutral pH to establish mild conditions for avoiding saponification of the lipids. Thus, the pH is preferably kept between 5 and 9, more preferably between 6 and 8, and very preferably between 6.5 and 8.0. - In case of a fermentation broth, out of convenience the fermentation broth may be employed, after the fermentation is ended, without any adjustment of the pH value.

[0044] If necessary or desired, the pH value may be adjusted by using bases or acids.

[0045] In general, adjusting the pH value can be carried out according to the invention by using either bases or acids as known to those skilled in the art. Decreasing of the pH can be carried out in particular by using organic or inorganic acids like sulfuric acid, nitric acid, phosphoric acid, boric acid, hydrochloric acid, hydrobromic acid, perchloric acid, hypochlorous acid, chlorous acid, fluorosulfuric acid, hexafluorophosphoric acid, acetic acid, citric acid, formic acid, or combinations thereof. As a high content of chloride is desirably avoided, in a preferred

embodiment of the invention no or only small amounts of hydrochloric acid are used in the process of the current invention. According to the invention, sulfuric acid is the preferred substance for decreasing the pH value. - Increasing of the pH can be carried out in particular by using organic or inorganic bases like hydroxides, in particular sodium hydroxide, lithium hydroxide, potassium hydroxide, and/or calcium hydroxide, carbonates, in particular sodium carbonate, potassium carbonate, or magnesium carbonate, and/or bicarbonates, in particular lithium bicarbonate, sodium bicarbonate, and/or potassium bicarbonate. - Due to easiness of handling, the acids and bases are preferably used in liquid form, in particular as concentrated solutions, wherein the concentration of acid or base in the solution is preferably in the range of 10 to 55 wt.-%, in particular in the range of 20 to 50 wt.-%. In particular sulfuric acid is preferably used also in concentrated form.

[0046] According to the invention after providing the suspension according to step (a) preferably a lysing step is carried out. The lysing step can be omitted, if - for example due to the fermentation conditions as applied - the cells or a big part thereof is already lysed or easily breakable in one of the following steps of the procedure without any explicit lysing step.

[0047] Lysing of the cells of the biomass according to step (b) can be carried out by methods as known to those skilled in the art, in particular enzymatically, mechanically, physically, or chemically, or by applying combinations thereof.

[0048] Depending on the time of exposure and/or the degree of force applied, a composition comprising only lysed cells or a composition comprising a mixture of cell debris and intact cells may be obtained. The term "lysed lipids containing biomass" insofar relates to a suspension which contains water, cell debris and oil as set free by the cells of the biomass, but beyond that may also comprise further components, in particular salts, intact cells, further contents of the lysed cells as well as components of a fermentation medium, in particular nutrients. In a preferred embodiment of the invention, only small amounts of intact cells, in particular less than 20 %, preferably less than 10 %, more preferably less than 5 % (relating to the total number of intact cells as present before lysing the cells of the biomass) are present in the lysed biomass after the step of lysing the cells.

[0049] Lysing of the cells may be realized for example by utilizing a French cell press, sonicator, homogenizer, microfluidizer, ball mill, rod mill, pebble mill, bead mill, high pressure grinding roll, vertical shaft impactor, industrial blender, high shear mixer, paddle mixer, and/or polytron homogenizer.

[0050] In a preferred embodiment of the invention, lysing of the cells comprises an enzymatic treatment of the cells by applying a cell-wall degrading enzyme.

[0051] According to the invention, the cell-wall degrading enzyme is preferably selected from proteases, cellulases (e.g., Cellustar CL (Dyadic), Fibrezyme G2000 (Dyadic), Celluclast (Novozymes), Fungamyl (Novozymes), Viscozyme L (Novozymes)), hemicellulases, chitinases, pectinases (e.g., Pectinex (Novozymes)), sucrases, maltases, lactases, alpha-glucosidases,

beta-glucosidases, amylases (e.g., Alphastar Plus (Dyadic); Termamyl (Novozymes)), lysozymes, neuraminidases, galactosidases, alpha-mannosidases, glucuronidases, hyaluronidases, pullulanases, glucocerebrosidases, galactosylceramidases, acetylgalactosaminidases, fucosidases, hexosaminidases, iduronidases, maltases-glucoamylases, xylanases (e.g., Xylanase Plus (Dyadic), Pentopan (Novozymes)), beta-glucanases (e.g., Vinoflow Max (Novozymes), Brewzyme LP (Dyadic)), mannanases, and combinations thereof. The protease may be selected from serine proteases, threonine proteases, cysteine proteases, aspartate proteases, metalloproteases, glutamic acid proteases, alcalases (subtilisins), and combinations thereof. The chitinase may be a chitotriosidase. The pectinase may be selected from pectolyases, pectozymes, polygalacturonases, and combinations thereof.

[0052] The adequate pH for utilizing the enzyme depends on the pH optimum of the enzyme.

[0053] In a preferred embodiment of the invention, an enzyme with a pH optimum of between 7.0 and 9.0, in particular of about 7.5, is used, so that the pH applied in this step is from 7.0 to 9.0, in particular from 7.0 to 8.0, preferably from 7.3 to 7.7. A preferred enzyme which can be used in this pH range is an alcalase.

[0054] The enzyme is preferably added as a concentrated enzyme solution, preferably in an amount of 0.01 to 1.5 wt.-%, more preferably in an amount of 0.03 to 1.0 wt.-%, above all in an amount of 0.05 to 0.5 wt.-%, relating to the amount of concentrated enzyme solution as added in relation to the total amount of the suspension after addition of the concentrated enzyme solution.

[0055] In a very preferred embodiment of the invention, lysing of the cells is carried out as follows:

1. i) Heating the suspension of (a) to a temperature of between 50°C and 70°C, preferably to a temperature of between 55°C and 65°C, and adding a cell wall-degrading enzyme to the suspension, in particular fermentation broth, and adjusting an adequate pH value, if necessary, at which the enzyme is properly working;
2. ii) Keeping the temperature and pH in the ranges as depicted in (ii) for at least one hour, preferably for at least two hours, more preferably for two to four hours.

[0056] In step (i), the enzyme can be added before or after heating up the suspension and/or before or after adjusting the pH. In the same way heating up of the suspension can be carried out before or after adjusting the pH. - But in a preferred embodiment, the enzyme is added after heating up of the suspension and after adjusting the pH, if adjusting of the pH is necessary, at all. - In a very preferred embodiment all measures are carried out more or less simultaneously.

[0057] Preferably, in the steps (i) and (ii) the suspension is continuously mixed by using a

stirrer and/or an agitator.

[0058] According to the invention, the demulsification is preferably carried out with a suspension having a dry matter content of 20 to 60 wt.-%, preferably 25 to 60 wt.%, in particular 30 to 55 wt.-% or 30 to 45 wt.-%. This can be realized by either providing a suspension with an appropriately high biomass in step (a) or by concentrating the suspension, in particular after lysing the cells of the biomass. Thus, in a preferred embodiment of the invention, after optional lysing of the cells of the biomass and before the demulsification step, the suspension is concentrated to a total dry matter content of 20 to 60 wt.-%, more preferably 25 to 60 wt.-%, in particular 30 to 55 wt.-%, above all 30 to 50 wt.-% or 30 to 45 wt.-%.

[0059] Concentration of the suspension is preferably carried out by evaporation of water at a temperature not higher than 100°C, preferably 70°C to 100°C, more preferably 80°C to 90°C, until a total dry matter content of 20 to 60 wt.-% more preferably 25 to 60 wt.-%, in particular 30 to 55 wt.-% or 30 to 45 wt.-%, is reached.

[0060] Concentration of the suspension is preferably carried out in a forced circulation evaporator (for example available from GEA, Germany) to allow fast removal of the water. Alternatively or in addition, concentration might be carried out by falling film evaporation, thin film evaporation and/or rotary evaporation.

[0061] The method according to the invention comprises as a further step the harvesting of the PUFAs containing lipid from the demulsified composition by separation of the oil containing light phase from the water, salts, cell debris and residual oil containing aqueous phase.

[0062] The harvesting of the PUFAs containing lipid may comprise as an optional step the neutralization of the demulsified suspension, if the demulsification was not carried out at neutral pH.

[0063] Neutralization of the demulsified composition is preferably realized, if necessary or desired, by adding an acid, preferably sulfuric acid, or base, preferably caustic soda, to adjust a pH value of 5 to 9, preferably 5.5 to 8.5, in particular 6.0 to 8.0 or 6.5 to 7.5. Before starting separation of the light phase from the aqueous phase, the neutralized composition may be stirred at this neutralized pH from several minutes up to several hours.

[0064] Separation of the oil containing light phase from the water, salts and cell debris containing heavy phase is preferably realized by mechanical means and preferably at a temperature of 60-90°C, more preferably 70-80°C, and preferably at a pH value of 6-9, more preferably 7-8.5. "Mechanical means" refers in particular to filtration and centrifugation methods as known to those skilled in the art.

[0065] After separation of the oil containing light phase, the PUFAs containing oil thus obtained can further be worked up by applying methods as known to those skilled in the art, in particular refining, bleaching, deodorizing and/or winterizing.

[0066] A particular advantage of the method of the current invention is that it can be carried out without the use of any organic solvent, in particular without the use of any polar or non-polar organic solvent. Thus, in a preferred embodiment of the invention, no or only little amounts of organic solvents, in particular of polar or non-polar organic solvents, are used for isolating the PUFAs containing oil from the biomass. Typical organic solvents are hexane and ethanol.

[0067] In a preferred embodiment of the invention less than 2 wt.-% non-polar organic solvents are used, more preferably less than 1, 0.5 or 0.1 wt.-%. In a particularly preferred embodiment of the invention no non-polar organic solvent is used, at all. In a very preferred embodiment of the invention less than 2 wt.-% organic solvents are used, in general, particularly preferred less than 1, 0.5 or 0.1 wt.-%. In a particularly very preferred embodiment of the invention no organic solvents are used, at all, for isolating the PUFAs containing oil from the biomass. - This means in particular for this embodiment that the suspension as employed in the method according to the invention as well as all compositions as obtained by said single method steps preferably contain non-polar organic solvents, preferably organic solvents in general, in an amount of less than 2 wt.-%, more preferably less than 1 wt.-%, in particular less than 0.5 or 0.3 wt.-%, above all in an amount of less than 0.1 or 0.05 wt.-%.

[0068] A further advantage of the method of the current invention is that a very effective separation of the oil from the remaining biomass can be realized without the addition of sodium chloride, which is normally used for salting out the oil from the biomass. Preferably the method can be carried out without the addition of chloride salts, at all, above all without the addition of any salts for salting out the oil. But small amounts of chloride salts, in particular sodium chloride, might be present in the suspension due to the fermentation medium as used for growing of the biomass.

[0069] Thus, in a preferred embodiment of the current invention, no or only little amounts of sodium chloride are used for improving the oil isolation. In a preferred embodiment of the invention less than 1 wt.-% of sodium chloride, are used, more preferably less than 0.5 or 0.2 wt.-% of sodium chloride are used for isolating the oil from the biomass, above all less than 0.1 or 0.05 wt.-%, wherein the wt.-% relate to the total weight of the composition after addition of the sodium chloride.

[0070] - This means in particular for this embodiment that the suspension as employed in the method according to the invention as well as all compositions as obtained by said single method steps preferably contain sodium chloride in an amount of less than 2 wt.-%, more preferably less than 1 wt.-%, in particular less than 0.5 or 0.3 wt.-%, above all in an amount of less than 0.1 or 0.05 wt.-%.

[0071] In a particularly preferred embodiment of the invention no or only little amounts of chloride salts are used for improving the oil isolation, at all. In this embodiment preferably less than 1 wt.-% of chloride salts, more preferably less than 0.5 or 0.2 wt.-% of chloride salts are

used for isolating the oil from the biomass, above all less than 0.1 or 0.05 wt.-%, wherein the wt.-% relate to the total weight of the composition after addition of the chloride salts. - This means in particular for this embodiment that the suspension as employed in the method according to the invention as well as all compositions as obtained by said single method steps preferably contain chloride, in particular chloride salts, in an amount of less than 2 wt.-%, more preferably less than 1 wt.-%, in particular less than 0.5 or 0.3 wt.-%, above all in an amount of less than 0.1 or 0.05 wt.-%.

[0072] In a very preferred embodiment of the invention no or only little amounts of salts are used for improving the oil isolation, in general. In this embodiment preferably less than 1 wt.-% of salts, more preferably less than 0.5 or 0.2 wt.-% of salts are used for isolating the oil from the biomass, above all less than 0.1 or 0.05 wt.-%, wherein the wt.-% relate to the total weight of the composition after addition of the salts. - This means that preferably, in particular for this embodiment of the invention, that the suspension as employed in the method according to the invention as well as all compositions as obtained by said single method steps preferably contain salts in general in an amount of less than 2 wt.-%, more preferably less than 1 wt.-%, in particular less than 0.5 or 0.3 wt.-%, above all in an amount of less than 0.1 or 0.05 wt.-%.

[0073] A further advantage of the method of the current invention is that a very efficient separation of the oil from the remaining biomass can be realized without the addition of bases like caustic soda, which are normally used for breaking the emulsion. Preferably the method can be carried out without the addition of bases, at all, so that also no subsequent neutralization of the suspension is needed. - This does result in a biomass which contains only relatively small amounts of salts, in general, and by that comprises only a small amount of ashes. But small amounts of bases might be added, in particular for adjusting the pH, for example if the pH of the suspension is too low after the fermentation, or to facilitate the demulsification process. - That means that in a preferred embodiment of the invention no or only little amounts of bases are used in the methods according to the invention. In a preferred embodiment of the invention less than 4 wt.-% of bases, are used, more preferably less than 2 or 1 wt.-% of bases are used in the methods according to the invention, above all less than 0.5 or 0.2 wt.-%, wherein the wt.-% relate to the total weight of the composition after addition of the base.

[0074] But in an alternative embodiment of the invention caustics, in particular caustic soda, may be added, preferably in small amounts, to facilitate the demulsification and to increase the final oil yield.

[0075] As the demulsification can be carried out without the use of bases, in a preferred embodiment of the invention, the pH can preferably kept during the complete procedure below a pH value of 9, in particular below 8.5, 8 or 7.5, which allows treatment of the lipid under very mild conditions and substantially reduces the degree of hydrolysis of the fatty acid esters as contained in the lipid. - Thus, in a preferred embodiment of the invention, the pH is kept during the complete procedure below 9, in particular in the range of 4.5 to 9, preferably below 8.5, 8 or 7.5, in particular in the range of 4.5 to 8.5, 4.5 to 8 or 4.5 to 7.5.

[0076] The methods of the current invention allow a very efficient separation of the oil contained in the biomass from the cell-debris and other substances as contained in the suspension, in particular fermentation broth. By using the methods of the current invention preferably more than 80 wt.-%, in particular more than 90 wt.-% of the oil contained in the biomass can be separated from the biomass and isolated by applying very economic and sustainable conditions.

[0077] The yield of liberated oil is preferably determined by fatty acid methyl ester analysis (FAME). For doing that the lipids in the sample are first saponified with KOH. After that, the free fatty acids are methylated with MeOH. The methylated fatty acids can then be determined and quantified by gas chromatography by using an internal standard.

[0078] It turned out further that the oil as obtained by applying the method of the current invention has some advantageous characteristics over the PUFAs containing oils as disclosed in the state of the art so far. In particular it exhibits very low oxidation values and a very low content of free fatty acids. Further, due to the method of preparation, the oil contains a certain amount of hydrophobic silica.

[0079] Thus, a further subject of the current invention is an oil as obtained or as obtainable by a method according to the current invention.

[0080] A further subject of the current invention is therefore also an oil, preferably a PUFAs containing oil, comprising hydrophobic silica in an amount of 5 ppm (w/w) to 10 wt.-%, preferably 10 ppm (w/w) to 5 wt.-%, in particular 0.01 wt.-% to 4 wt.-%, 0.1 wt.-% to 3 wt.-% or 0.5 wt.-% to 2.5 wt.-%.

[0081] In a very preferred embodiment only small amounts of hydrophobic silica are used for breaking the emulsion, so that a particular embodiment of the invention is a PUFAs containing oil, comprising hydrophobic silica in an amount of 5 to 200 ppm (w/w), preferably 10 to 150 ppm (w/w), more preferably 20 to 100 ppm (w/w), in particular 20 to 80 ppm (w/w).

[0082] The oil according to the invention preferably comprises PUFAs in an amount of at least 10 wt.-%, preferably at least 20 wt.-%, in particular 25 to 70 wt.-%, 30 to 65 wt.-% or 45 to 60 wt.-%. The oil according to the invention preferably comprises free fatty acids (FFA) in an amount of less than 1 wt.-%, preferably less than 0.8 wt.-%, more preferably less than 0.5 wt.-%. Free fatty acids are undesirable by-products in the isolation of TAGs containing lipids, which preferably are avoided.

[0083] A further subject of the current invention is therefore also an oil, in particular a PUFAs containing oil, which comprises fatty acid esters, in particular TAGs, in an amount of at least 50 wt.-%, preferably at least 70 or 80 wt.-%, in particular in an amount of at least 85 or 90 wt.-%, characterized by comprising free fatty acids (FFAs) in an amount of less than 0.6 wt.-%, preferably in an amount of less than 1 wt.-%, in particular in an amount of less than 0.8 wt.-%,

more preferably in an amount of less than 0.5 wt.-%.

[0084] A further subject is therefore also a PUFAs containing oil exhibiting the following characteristics: a) hydrophobic silica in an amount of 5 ppm to 10 wt.-%, preferably 10 ppm to 5 wt.-%, in particular 0.01 wt.-% to 4 wt.-%, 0.1 wt.-% to 3 wt.-% or 0.5 wt.-% to 2.5 wt.-%; b) preferably a content of free fatty acids of less than 1 wt.-%, preferably less than 0.8 wt.-%, in particular less than 0.5 wt.-%; c) preferably a peroxide value of less than 0.5, more preferably less than 0.3, in particular less than 0.15; d) preferably an anisidine value of less than 15, more preferably less than 10; e) preferably a content of moisture and impurities of less than 1 wt.-%, preferably less than 0.5 wt.-%; f) preferably a viscosity of less than 250 cps, more preferably of less than 200 cps, in particular of less than 160 cps; g) preferably a flash point of at least 350°C, more preferably of at least 400°C, in particular of at least 450°C; h) preferably a content of omega-3 fatty acids, in particular of DHA and EPA, of at least 35 wt.-%, preferably at least 40 or 45 wt.-%, above all at least 50 wt.-%; i) preferably DHA and EPA each in an amount of at least 8 wt.-%, preferably at least 10 wt.-%, above all at least 15 wt.-%; j) preferably a content of triacylglycerols of at least 50 wt.-%, in particular at least 70 or 80 wt.-%, more preferably at least 90 wt.-%; k) preferably an amount of organic solvents of less than 0.5 wt.-%, more preferably less than 0.1 wt.-%, in particular less than 0.05 wt.-%, above all less than 0.01 wt.-%; l) preferably an amount of chlorides of less than 0.1 wt.-%, more preferably less than 0.05 wt.-%, in particular less than 0.01 wt.-%; m) preferably a content of crude fat of more than 90 wt.-%.

[0085] The anisidine value (AV) is determined in accordance with AOCS Official Method Cd 18-90. The AV is a measure for secondary reaction products of the fatty acids, such as aldehydes and ketones, that occur during oxidation of the oil.

[0086] The peroxide value (PV) is determined in accordance with the AOCS Official Method CD 8-53. The PV is a measure for primary reaction products, such as peroxide and hydroperoxides, that occur during oxidation of the oil. - According to the invention the PV is measured in meq/kg.

[0087] The content of free fatty acids is determined in accordance with AOCS Official Method AOCS Ca 5a-40. The content of moisture is determined in accordance with AOCS Official Methods AOAC 930.15, 935.29. The content of insoluble impurities is determined in accordance with AOCS Official Method AOCS 3a-46. The amount of DHA and EPA is determined in accordance with AOCS Official Method AOCS Ce 1b-89. The amount of total fat is determined in accordance with AOCS Official Method AOCS 996.06. The amount of crude fat is determined in accordance with AOCS Official Methods AOAC 920.39, 954.02.

[0088] As the isolation of the oil is carried out by using no or only small amounts of solvents and by also using no or only small amounts of sodium chloride, the aqueous phase obtained as a by-product is preferably substantially free of organic solvents and sodium chloride, as well. Thus, the aqueous phase can be utilized in different ways, either directly after separation of the oil phase or after further work-up like concentrating and/or drying.

[0089] A further subject is therefore a lipids, in particular PUFAs, containing aqueous suspension, containing a biomass, preferably a delipidated biomass, as obtained or as obtainable by a method according to the current invention. A further subject is therefore also a concentrate or a dried product as obtained or obtainable by concentrating and/or drying this aqueous suspension. When concentrating the aqueous suspension, it is preferably dried until a total dry matter (TDM) content of 20 to 60 wt.-% is reached. - In the following the expression "aqueous suspension according to the invention" refers to the aqueous phase as obtained after separation of the oil phase as well as to any concentrated suspensions of this aqueous phase as obtained by concentrating this aqueous phase. Drying is preferably carried out by solvent evaporation, as described further below.

[0090] A further subject of the current invention is therefore also a lipids, in particular PUFAs, containing aqueous suspension, containing a biomass, in particular cell debris of a delipidated biomass, characterized by a content of salts of less than 15 wt.-%, preferably 4 to 12 wt.-%, in particular 6 to 10 wt.-%, and characterized further by a content of non-polar organic solvents of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%, above all less than 0.01 wt.-%, and preferably further characterized by a content of chloride ions of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0091] A further subject is therefore in particular also a lipids, in particular PUFAs, containing aqueous suspension, containing a biomass, in particular, cell debris of a delipidated biomass, characterized by a content of salts of less than 15 wt.-%, preferably 4 to 12 wt.-%, in particular 6 to 10 wt.-%, and preferably characterized further by a content of organic solvents of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%, above all less than 0.01 wt.-%, and preferably further characterized by a content of chloride ions of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0092] A preferred subject of the current invention is therefore also a lipids, in particular PUFAs, containing aqueous suspension, containing a Thraustochytrid biomass, in particular cell debris of a delipidated Thraustochytrid biomass, characterized by a content of salts of less than 15 wt.-%, preferably 4 to 12 wt.-%, in particular 6 to 10 wt.-%, and characterized by a content of non-polar organic solvents of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%, above all less than 0.01 wt.-%, and preferably further characterized by a content of chloride ions of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0093] A particularly preferred subject of the current invention is therefore also a lipids, in particular PUFAs, containing aqueous suspension, containing a Thraustochytrid biomass, in particular, cell debris of a delipidated Thraustochytrid biomass, characterized by a content of salts of less than 15 wt.-%, preferably 4 to 12 wt.-%, in particular 6 to 10 wt.-%, and preferably characterized by a content of organic solvents of less than 1 wt.-%, preferably less than 0.5 or

0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%, above all less than 0.01 wt.-%, and further characterized by a content of chloride ions of less than 1 wt.-%, preferably less than 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0094] The aqueous suspensions of the invention as described before preferably exhibit a total dry matter (TDM) content of 20 to 60 wt.-%, in particular of 25 to 55 wt.-%, more preferably of 30 to 50 wt.-%, as such concentrated suspensions turned out as particularly suitable for the applications of the invention as described below.

[0095] The aqueous suspensions of the invention can be used as a product for different purposes as disclosed further below. - Alternatively the aqueous suspension may also be worked up further to increase the overall oil yield. The further work-up and isolation of further oil may be carried out by using caustic soda.

[0096] "Chloride" according to the invention refers to the amount of detectable chlorine. The amount of chlorine as present can be determined for example by elemental analysis according to DIN EN ISO 11885. The chlorine is present in the form of salts which are called "chlorides". The content of chloride as mentioned according to the invention - also called "chloride ions" - only refers to the amount of detectable chlorine, not to the amount of the complete chloride salt, which comprises besides the chloride ion also a cationic counterion.

[0097] The total dry matter content (TDM) is preferably determined by gravimetric analysis. For doing that, a sample of the homogeneous suspension with a specific volume is weighed before and after freeze-drying. The remaining weight of the dried sample corresponds to the total dry matter as contained in that specific volume of the suspension.

[0098] In a particularly preferred embodiment of the current invention the water, salts, residual oil and cell debris containing aqueous phase, which is obtained as by-product in the oil harvesting step as described before, is converted into a dried biomass by drying the biomass to a total dry matter content of more than 90 wt.-%.

[0099] Due to the method of manufacture, the biomass comprises a very low content of salts, preferably ashes, of less than 30 wt.-%, in particular 15 to 20 wt.-%.

[0100] Thus, a further subject of the current invention is also a lipids, preferably PUFAs, containing biomass, in particular a delipidated biomass, characterized by a content of ashes, in particular salts, of less than 30 wt.-%, preferably 8 to 30 wt.-%, more preferably 12 to 20 wt.-%, in particular 15 to 20 wt.-%.

[0101] The delipidated biomass, according to the inventon is preferably characterized by a content of non-polar organic solvents of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1, 0.05 or 0.02 wt.-% and preferably further characterized by a content of chloride ions of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0102] Thus, a preferred subject of the current invention is also a lipids, in particular PUFAs, containing Thraustocyhtrid biomass, in particular a delipidated Thraustochytrid biomass, characterized by a content of ashes, in particular salts, of less than 30 wt.-%, preferably 8 to 30 wt.-%, more preferably 12 to 20 wt.-%, in particular 15 to 20 wt.-%, and preferably a content of non-polar organic solvents of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1, 0.05 or 0.02 wt.-% and preferably further characterized by a content of chloride ions of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0103] Thus, a particularly preferred subject of the current invention is also a lipids, in particular PUFAs, containing Thraustocyhtrid biomass, in particular a delipidated Thraustochytrid biomass, characterized by a content of ashes, in particular salts, of less than 30 wt.-%, preferably 8 to 30 wt.-%, more preferably 12 to 20 wt.-%, in particular 15 to 20 wt.-%, and preferably a content of organic solvents of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1, 0.05 or 0.02 wt.-% and preferably further characterized by a content of chloride ions of less than 2 wt.-%, preferably less than 1, 0.5 or 0.2 wt.-%, more preferably less than 0.1 or 0.05 wt.-%.

[0104] As preferably the preparation is carried out without the use of non-polar organic solvents, preferably without the use of any organic solvents, at all, and without the use of sodium chloride, preferably without the use of chloride salts, at all, the resulting biomass is preferably free of any non-polar organic solvents, preferably free of any organic solvents, in general, and further essentially free of any chloride ions, at all, wherein "essentially free" means that it contains chloride ions in an amount of less than 0.1 wt.-%, in particular in an amount of less than 0.05 wt.-%.

[0105] The biomass according to the invention exhibits preferably a moisture content of not more than 10 wt.-%, preferably not more than 5 wt.-%.

[0106] The biomass thus obtained preferably comprises lipids (crude fat) in an amount of about 3 to 14 wt.-%, in particular about 4 to about 14 wt.-%, preferably in an amount of about 4.5 to about 12 wt.-%, more preferably in an amount of about 5 to about 10 wt.-%. Further, the lipid preferably comprises at least one PUFA selected from DHA and EPA, more preferably a mixture of DHA and EPA, wherein the ratio of DHA to EPA is preferably between 3:2 to 4:1 and wherein the amount of DHA is preferably from 30 to 50 wt.-% of the total amount of lipids contained and the amount of EPA is preferably from 10 to 20 wt.-%. of the total amount of lipids contained. Accordingly, also the aqueous suspensions as described before are preferably characterized by being convertible by drying into a biomass with such a crude fat content and/or EPA content and/or DHA content by drying the aqueous suspension to a moisture content of not more than 10 wt.-%, preferably not more than 5 wt.-%.

[0107] The biomass preferably further comprises proteins and/or amino acids in an amount of 15 to 25 wt.-%, more preferably in an amount of 17 to 23 wt.-%, and exhibits preferably a

crude protein content of 25 to 35 wt.-%. As the demulsification is carried out under mild conditions, the weight ratio of free amino acids to the sum of proteins and peptides is preferably less than 9:1, more preferably less than 5:1, in particular less than 1:1. Accordingly, also the aqueous suspensions as described before are preferably characterized by being convertible by drying into a biomass with such an amino acid and/or crude protein content and/or ration of free amino acids to proteins and peptides by drying the aqueous suspension to a moisture content of not more than 10 wt.-%, preferably not more than 5 wt.-%.

[0108] The biomass preferably further exhibits a crude fiber content of less than 5 wt.-%, preferably less than 2 wt.-%, more preferably of about 0 wt.-%. Accordingly, also the aqueous suspensions as described before are preferably characterized by being convertible by drying into a biomass with such a crude fiber content by drying the aqueous suspension to a moisture content of not more than 10 wt.-%, preferably not more than 5 wt.-%.

[0109] The dried biomass is preferably a delipidated biomass, that means a biomass, of which the major part of the lipids have been removed, preferably by a process as disclosed in this application. As the separation of oil from the biomass is very effectively, the remaining oil in the biomass is preferably less than 20 wt.-%, preferably less than 15 wt.-%, more preferably less than 10 wt.-%, of the oil as originally contained in the biomass. But as the oil cannot be removed completely by such a process, a substantial amount of oil is still contained also in the delipidated biomass according to the invention. That means that the term "delipidated biomass" according to the invention refers to a lysed biomass, from which the major part of oil has been removed, preferably by a process or method as disclosed in this application, but which still contains a substantial part of lipids, in particular of PUFAs containing lipids, wherein the amount of lipids in the dried delipidated biomass is preferably from 3-14 wt.-%, in particular 4-14 wt.-%, preferably from 4.5-12 wt.-%, more preferably from 5-10 wt.-%. Thus, the "delipidated biomass" according to the invention might also be called a "partially delipidated biomass" or a "substantially delipidated biomass".

[0110] Thus, a further subject is a method of obtaining a biomass which has a low content of salts, is substantially free of non-polar organic solvents, preferably free of organic solvents, in general, and which is further substantially free of sodium chloride, preferably free of chloride salts, in general, comprising the method steps as mentioned before.

[0111] Conversion of the water, salts, remaining oil and cell debris containing heavy phase, which is obtained as by-product in the oil harvesting step, into a dried biomass by drying the biomass to a total dry matter content of more than 90 wt.-%, can be carried out in different ways.

[0112] In a very preferred way, the transformation is carried out by concentration of the heavy phase to a dry matter content of 30-50 wt.-%, preferably 35-45 wt.-%, and subsequent spray granulation of the biomass by means of fluidized bed granulation. By doing that, in a very efficient way, a biomass with advantageous features can be obtained. Spray granulation by means of fluidized bed granulation is disclosed in more detail in EP13176661.0.

[0113] Concentration of the heavy phase to a dry matter content of 30-50 wt.-% is preferably carried out by solvent evaporation, in particular vacuum evaporation, and/or by using a rotary evaporator, a thin-film evaporator or a falling-film evaporator. A useful alternative to solvent evaporation is reverse osmosis.

[0114] As alternative to the spray-granulation other drying methods, in particular other convective drying methods, like tunnel drying or spray drying, in particular nozzle spray drying, or contact drying methods, like drum drying, or radiation drying methods, like infrared drying, of the concentrated heavy phase would be applicable alternatives, wherein by using those methods normally particles with a smaller or bigger diameter are obtained.

[0115] According to the invention, during the drying process, an anti-caking agent, in particular silica, preferably a hydrophobic or hydrophilic silica, may optionally be added to the biomass to prevent caking. For this purpose, the suspension, in particular fermentation broth, comprising biomass as well as the silica are preferably sprayed into the particular drying zone. Alternatively or additionally, the biomass may be mixed with the anti-caking agent after the drying process. With respect to the use of silica as anti-caking agent reference is made in particular to the patent application EP13187631.0.

[0116] Conversion of a fine-grained powder into a coarse-grained dust-free product can be realized by granulating processes. Conventional organic or inorganic auxiliaries or supports such as starch, gelatin, cellulose derivatives or similar substances, which are typically used in food processing or feed processing as binding agents, gelling agents or thickeners, may optionally be used in this subsequent granulation process. Further auxiliaries that are preferably used according to the invention are disclosed in WO 2016/050560, with carboxymethylcellulose being a particulary preferred binding agent.

[0117] After drying and optionally granulating and/or sieving of the biomass, the dried biomass is preferably stored or packed.

[0118] The particulate biomass of the invention as well as the aqueous suspensions of the invention can be used in different ways. For example, they can be used in order to produce a foodstuff or feedstuff. Alternatively they may be used directly as foodstuff or feedstuff.

[0119] A further subject is therefore likewise a method for producing a feedstuff or foodstuff, in which a particulate biomass and/or an aqueous suspension according to the invention is used, and is preferably mixed with further feedstuff or foodstuff ingredients.

[0120] In a preferred embodiment the particulate biomass and/or the aqueous suspension is used for producing a foodstuff or feedstuff, in which the biomass and/or the aqueous suspension is preferably mixed with other foodstuff or feedstuff ingredients and is then processed to give the foodstuff or feedstuff.

[0121] The mixture of biomass and/or aqueous suspension and other foodstuff or feedstuff ingredients is processed in a preferred embodiment by an extrusion process, in order to obtain portions of foodstuff or feedstuff ready for sale. Alternatively, a pelleting method may also be used.

[0122] A screw or twin-screw extruder is preferably employed in the extrusion process. The extrusion process is preferably carried out at a temperature of 80 - 220°C, particularly 100 - 190°C, a pressure of 10 - 40 Bar, and a shaft rotational speed of 100 - 1000 rpm, particularly 300 - 700 rpm. The residence time of the mixture introduced is preferably 5-30 seconds, in particular 10 - 20 seconds.

[0123] In a mode of the extrusion process which is preferred in accordance with the invention, the process comprises a compacting step and a compression step.

[0124] It is preferred to intimately mix the components with each other before carrying out the extrusion process. This is preferably carried out in a drum equipped with vanes. In this mixing step, a preferred embodiment includes an injection of steam, in particular so as to bring about the swelling of the starch which is preferably present.

[0125] Before being mixed with the biomass and/or aqueous suspension, the further foodstuff or feedstuff ingredients are preferably comminuted - if required - so as to ensure that a homogeneous mixture is obtained in the mixing step. The comminuting of the further foodstuff or feedstuff ingredients may be carried out, for example, using a hammer mill.

[0126] A further subject of the current invention is therefore a method of feeding animals, wherein a particulate biomass and/or an aqueous suspension according to the invention are provided to animals, preferably after mixing the particulate biomass and/or the aqueous suspension with further feedstuff ingredients, wherein the animals are preferably selected from poultry, swine, minks, ruminants, in particular from calves and beef cattle, sheep, goats, companion animals or animals hold in aquaculture.

[0127] Alternatively the biomass and/or aqueous suspension according to the invention may be used in land applications, in particular as (organic) fertilizer, NPC (nitrogen/phosphorous/potassium source), soil enhancer, plant enhancer and/or composting aid, for producing biogas, for wastewater treatment or as alternative fuel, in particular for cement kilns. It might be further used as part of a fermentation medium for producing microorganisms, in particular for producing further PUFAs containing biomass.

[0128] A further subject is therefore a method for enhancing soil, wherein a particulate biomass and/or an aqueous suspension according to the invention are strewed on and possibly mixed with ground, in particular with farmland soil or garden soil.

[0129] A further subject is therefore also a method for fertilizing and/or composting ground, in particular farmland or garden, wherein a particulate biomass and/or an aqueous suspension

according to the invention are strewed on and possibly mixed with ground, in particular with farmland soil or garden soil.

[0130] A further subject is therefore also a method for producing biogas, wherein a particulate biomass and/or an aqueous suspension according to the invention is subjected to microbial degradation under anaerobic conditions, in particular by making use of methanogenic bacteria.

[0131] A further subject of the current invention is therefore also a method for treatment of wastewater, wherein wastewater is mixed with a particulate biomass and/or an aqueous suspension according to the invention.

[0132] A further subject is therefore also a method for producing microorganisms, in particular for producing a PUFAs containing biomass, wherein a particulate biomass and/or aqueous suspension according to the invention is used as part of the fermentation medium.

[0133] The lipids containing cells according to the invention contain in average at least 10 wt.-% of lipids, preferably at least 20 or 30, more preferably at least 40 or 50 wt.-%, of lipids.

[0134] The lipids containing cells according to the invention preferably further contain polyunsaturated fatty acids (PUFAs).

[0135] The lipids, and in particular PUFAs, containing cells of the biomass are preferably microbial cells or plant cells. In a preferred embodiment of the invention, the cells are capable of producing the PUFAs due to a polyketide synthase system. The polyketide synthase system may be an endogenous one or, due to genetic engineering, an exogenous one.

[0136] Accordingly, "delipidated biomass" according to the invention refers in particular to the residues of such a PUFAs containing cells comprising biomass, in particular as disclosed further below, after having been subjected to an oil isolation process, in particular as disclosed further before.

[0137] The plant cells may in particular be selected from cells of the families Brassicaceae, Elaeagnaceae and Fabaceae. The cells of the family Brassicaceae may be selected from the genus Brassica, in particular from oilseed rape, turnip rape and Indian mustard; the cells of the family Elaeagnaceae may be selected from the genus Elaeagnus, in particular from the species Oleae europaea; the cells of the family Fabaceae may be selected from the genus Glycine, in particular from the species Glycine max.

[0138] The microbial organisms which contain a PUFAs containing lipid are described extensively in the prior art. The cells used may, in this context, in particular be cells which already naturally produce PUFAs (polyunsaturated fatty acids); however, they may also be cells which, as the result of suitable genetic engineering methods or due to random mutagenesis, show an improved production of PUFAs or have been made capable of producing PUFAs, at all. The production of the PUFAs may be auxotrophic, mixotrophic or

heterotrophic.

[0139] The biomass preferably comprises cells which produce PUFAs heterotrophically. The cells according to the invention are preferably selected from algae, fungi, particularly yeasts, bacteria, or protists. The cells are more preferably microbial algae or fungi.

[0140] Suitable cells of oil-producing yeasts are, in particular, strains of Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces.

[0141] Suitable cells of oil-producing microalgae and algae-like microorganisms are, in particular, microorganisms selected from the phylum Stramenopiles (also called Heterokonta). The microorganisms of the phylum Stramenopiles may in particular be selected from the following groups of microorganisms: Hamatores, Proteromonads, Opalines, Developayella, Diplophrys, Labrinthulids, Thraustochytrids, Biosecids, Oomycetes, Hypochytridiomycetes, Commation, Reticulosphaera, Pelagomonas, Pelagococcus, Ollicola, Aureococcus, Parmales, Diatoms, Xanthophytes, Phaeophytes (brown algae), Eustigmatophytes, Raphidophytes, Synurids, Axodines (including Rhizochromulinales, Pedinellales, Dictyochales), Chrysomeridales, Sarcinochrysidales, Hydrurales, Hibberdiales, and Chromulinales. Other preferred groups of microalgae include the members of the green algae and dinoflagellates, including members of the genus Crypthecodiurn.

[0142] The biomass according to the invention preferably comprises cells, and preferably consists essentially of such cells, of the taxon Labyrinthulomycetes (Labyrinthulea, net slime fungi, slime nets), in particular those from the family of Thraustochytriaceae. The family of the Thraustochytriaceae (Thraustochytrids) includes the genera Althomia, Aplanochytrium, Aurantiochytrium, Botryochytrium, Elnia, Japonochytrium, Oblongichytrium, Parietichytrium, Schizochytrium, Sicyoidochytrium, Thraustochytrium, and Ulkenia. The biomass particularly preferably comprises cells from the genera Aurantiochytrium, Oblongichytrium, Schizochytrium, or Thraustochytrium, above all from the genus Schizochytrium.

[0143] In accordance with the invention, the polyunsaturated fatty acid (PUFA) is preferably a highly-unsaturated fatty acid (HUFA).

[0144] The cells present in the biomass are preferably distinguished by the fact that they contain at least 20% by weight, preferably at least 30% by weight, in particular at least 35% by weight, of PUFAs, in each case based on cell dry matter.

[0145] According to the current invention, the term "lipid" includes phospholipids; free fatty acids; esters of fatty acids; triacylglycerols; sterols and sterol esters; carotenoids; xanthophylls (e. g. oxycarotenoids); hydrocarbons; isoprenoid-derived compounds and other lipids known to one of ordinary skill in the art. - The terms "lipid" and "oil" are used interchangeably according to the invention.

[0146] In a preferred embodiment, the majority of the lipids in this case is present in the form

of triglycerides, with preferably at least 50% by weight, in particular at least 75% by weight and, in an especially preferred embodiment, at least 90% by weight of the lipids present in the cell being present in the form of triglycerides.

[0147] According to the invention, polyunsaturated fatty acids (PUFAs) are understood to mean fatty acids having at least two, particularly at least three, C-C double bonds. According to the invention, highly-unsaturated fatty acids (HUFAs) are preferred among the PUFAs. According to the invention, HUFAs are understood to mean fatty acids having at least four C-C double bonds.

[0148] The PUFAs may be present in the cell in free form or in bound form. Examples of the presence in bound form are phospholipids and esters of the PUFAs, in particular monoacyl-, diacyl- and triacylglycerides. In a preferred embodiment, the majority of the PUFAs is present in the form of triglycerides, with preferably at least 50% by weight, in particular at least 75% by weight and, in an especially preferred embodiment, at least 90% by weight of the PUFAs present in the cell being present in the form of triglycerides.

[0149] Preferred PUFAs are omega-3 fatty acids and omega-6 fatty acids, with omega-3 fatty acids being especially preferred. Preferred omega-3 fatty acids here are the eicosapentaenoic acid (EPA, 20:5ω-3), particularly the (5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17-pentaenoic acid, and the docosahexaenoic acid (DHA, 22:6ω-3), particularly the (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid.

[0150] In a very preferred embodiment of the current invention, cells, in particular a Schizochytrium strain, is employed which produces a significant amount of EPA and DHA, simultaneously, wherein DHA is preferably produced in an amount of at least 20 wt.-%, preferably in an amount of at least 30 wt.-%, in particular in an amount of 30 to 50 wt.-%, and EPA is produced in an amount of at least 5 wt.-%, preferably in an amount of at least 10 wt.-%, in particular in an amount of 10 to 20 wt.-% (in relation to the total amount of lipid as contained in the cells, respectively). DHA and EPA producing *Schizochytrium* strains can be obtained by consecutive mutagenesis followed by suitable selection of mutant strains which demonstrate superior EPA and DHA production and a specific EPA:DHA ratio. Any chemical or nonchemical (e.g. ultraviolet (UV) radiation) agent capable of inducing genetic change to the yeast cell can be used as the mutagen. These agents can be used alone or in combination with one another, and the chemical agents can be used neat or with a solvent.

[0151] Preferred species of microorganisms of the genus Schizochytrium, which produce EPA and DHA simultaneously in significant amounts, as mentioned before, are deposited under ATCC Accession No. PTA-10208, PTA-10209, PTA-10210, or PTA-10211, PTA-10212, PTA-10213, PTA-10214, PTA-10215.

[0152] The suspension of biomass according to the present invention is preferably a fermentation broth. The suspension, in particular the fermentation broth, has preferably a biomass density of at least 80 or 100 g/l, in particular of 80 or 100 g/l to 250 g/l, preferably at

least 120 or 140 g/l, in particular 120 g/l or 140 g/l to 220 g/l, more preferably at least 160 or 180 g/l, in particular 160 g/l to 200 g/l (calculated as dry-matter content). Thus, the suspension may be obtained by culturing and growing suitable cells in a fermentation medium under conditions whereby the PUFAs are produced by the microorganism.

[0153] Methods for producing the biomass, in particular a biomass which comprises cells containing lipids, in particular PUFAs, particularly of the order Thraustochytriales, are described in detail in the prior art (see e.g. WO91/07498, WO94/08467, WO97/37032, WO97/36996, WO01/54510). As a rule, the production takes place by cells being cultured in a fermenter in the presence of a carbon source and of a nitrogen source, along with a number of additional substances like minerals that allow growth of the microorganisms and production of the PUFAs. In this context, biomass densities of more than 100 grams per litre and production rates of more than 0.5 gram of lipid per litre per hour may be attained. The process is preferably carried out in what is known as a fed-batch process, i.e. the carbon and nitrogen sources are fed in incrementally during the fermentation. When the desired biomass has been obtained, lipid production may be induced by various measures, for example by limiting the nitrogen source, the carbon source or the oxygen content or combinations of these.

[0154] In a preferred embodiment of the current invention, the cells are grown until they reach a biomass density of at least 80 or 100 g/l, in particular of 80 or 100 g/l to 250 g/l, preferably at least 120 or 140 g/l, in particular 120 g/l or 140 g/l to 220 g/l, more preferably at least 160 or 180 g/l, in particular 160 g/l to 200 g/l (calculated as dry-matter content). Such processes are for example disclosed in US 7,732,170.

[0155] Preferably, the cells are fermented in a medium with low salinity, in particular so as to avoid corrosion. This can be achieved by using chlorine-free sodium salts as the sodium source instead of sodium chloride, such as, for example, sodium sulfate, sodium carbonate, sodium hydrogen carbonate or soda ash. Preferably, chloride is used in the fermentation in amounts of less than 3 g/l, in particular less than 500 mg/l, especially preferably less than 100 mg/l.

[0156] Suitable carbon sources are both alcoholic and non-alcoholic carbon sources. Examples of alcoholic carbon sources are methanol, ethanol and isopropanol. Examples of non-alcoholic carbon sources are fructose, glucose, sucrose, molasses, starch and corn syrup.

[0157] Suitable nitrogen sources are both inorganic and organic nitrogen sources. Examples of inorganic nitrogen sources are nitrates and ammonium salts, in particular ammonium sulphate and ammonium hydroxide. Examples of organic nitrogen sources are amino acids, in particular glutamate, and urea.

[0158] In addition, inorganic or organic phosphorus compounds and/or known growth-stimulating substances such as, for example, yeast extract or corn steep liquor, may also be added so as to have a positive effect on the fermentation.

[0159] The cells are preferably fermented at a pH of 3 to 11, in particular 4 to 10, and preferably at a temperature of at least 20°C, in particular 20 to 40°C, especially preferably at least 30°C. A typical fermentation process takes up to approximately 100 hours.

[0160] After the fermentation has ended, the cells may be pasteurized in order to kill the cells and to deactivate enzymes which might promote lipid degradation. The pasteurization is preferably effected by heating the biomass to a temperature of 50 to 121°C, preferably 50 to 70°C, for a period of 5 to 150 minutes, in particular 20 to 100 minutes.

[0161] Likewise, after the fermentation is ended, antioxidants may be added in order to protect the PUFAs present in the biomass from oxidative degradation. Preferred antioxidants in this context are BHT, BHA, TBHA, ethoxyquin, beta-carotene, vitamin E, in particular tocopherol, and vitamin C. The antioxidant, if used, is preferably added in an amount of 0.001 to 0.1 wt.-%, preferably in an amount of 0.002 to 0.05 wt.-%, relating to the total amount of the fermentation broth after addition of the antioxidant.

Working examples

Example 1: Preparation of the lysed and concentrated fermentation broth

[0162] An unwashed cell broth containing microbial cells (Schizochytrium sp.) at a biomass density of over 100 g/l was heated to 60°C in an agitated vessel. After heating up the suspension, the pH was adjusted to 7.5 by using caustic soda (50 wt.-% NaOH solution), before an alcalase (Alcalase® 2.4 FG (Novozymes)) was added in liquid form in an amount of 0.5 wt.-% (by weight broth). Stirring was continued for 3 hours at 60°C. After that, the lysed cell mixture was transferred into a forced circulation evaporator (obtained from GEA, Germany) and heated to a temperature of 85°C. The mixture was concentrated in the forced circulation evaporator, until a total dry matter content of about 35 wt.-% was reached.

Example 2: Demulsification using a mixture of hydrophobic silica and surfactants

[0163] To a sample of 1000 g of the lysed and concentrated fermentation broth as obtained according to example 1 were added 5 g of a demulsifier mixture containing 47.5 wt.-% Tween 80, 47.5 wt.-% Span 80 and 5 wt.-% of a hydrophobic silica selected from Sipernat® D10, Sipernat® D17 and Aerosil® 202 R (Evonik Industries, Germany). - As negative control 5 g of the mixture of Tween 80 and Span 80 was used without addition of hydrophobic silica. - The demulsifier mixture was prepared by thoroughly mixing the components before applying the mixture.

[0164] The suspension thus obtained, after addition of the demulsifier mixture, was heated in an agitated vessel to a temperature of 90°C. Samples of 100 ml were taken after 1 and 22 hours, respectively, and oil was subsequently separated from the aqueous phase by centrifugation.

[0165] The results are disclosed in the following table.

Table 1: Demulsification using a mixture of hydrophobic silica and surfactants

Hydrophobic surfactant	Yield after one hour	Yield after 22 hours	FFA after 22 hours [wt.-%]
Sipernat D10	83.5 %	86.8 %	0.60
Sipernat D17	83.2 %	92.1 %	0.60
Aerosil 202 R	86.3 %	87.0 %	0.55
none	No free oil	No free oil	------

[0166] As can be seen a high yield of oil can be obtained already after one hour of incubation, but the yield is higher after 22 hours of incubation.

Example 3: Demulsification using different amounts of demulsifier mixture

[0167] To samples of 1000 g of the lysed and concentrated fermentation broth as obtained according to example 1 were added 1, 3, 5 or 8 g of a demulsifyer mixture containing 47.5 wt.-% Tween 80, 47.5 wt.-% Span 80 and 5 wt.-% Sipernat® D17 (Evonik Industries, Germany), corresponding to a final amount of hydrophobic silica in the suspension of 0.005, 0.015, 0.025 and 0.040 wt.-%, respectively.

[0168] The suspensions thus obtained were heated in an agitated vessel to a temperature of 90°C. Samples of 100 ml were taken after 24 hours, respectively, and oil was subsequently separated from the aqueous phase by centrifugation.

[0169] The results are disclosed in the following table.

Table 2: Demulsification using a mixture of hydrophobic silica and surfactants

Amount of demulsifyer mixture	Amount of hydrophobic silica in aqeous suspension	Yield after 24 hours	FFA after 24 hours [wt.-%]
0.1 wt.-%	0.005 wt.-%	87.4 %	0.60
0.3 wt.-%	0.015 wt.-%	87.1 %	0.70
0.5 wt.-%	0.025 wt.-%	89.0 %	0.70
0.8 wt.-%	0.040 wt.-%	86.6 %	0.75

[0170] As can be seen, an efficient demulsification takes place already by adding an amount of demulsifier mixture of 1 g per kg suspension, corresponding to an amount of hydrophobic silica in the suspension of 0.005 wt.-%.

Example 4: Demulsification at different incubation times

[0171] To a sample of 1000 g of the lysed and concentrated fermentation broth as obtained according to example 1 were added 5 g of a demulsifier mixture containing 47.5 wt.-% Tween 80, 47.5 wt.-% Span 80 and 5 wt.-% Sipernat® D17 (Evonik Industries, Germany), corresponding to a final amount of hydrophobic silica in the suspension of 0.025 wt.-%.

[0172] The suspension thus obtained was heated in an agitated vessel to a temperature of 90°C. Samples of 100 ml were taken after 2, 4, 6 and 24 hours, respectively, and oil was subsequently separated from the aqueous phase by centrifugation.

[0173] The results are disclosed in the following table.

Table 3: Demulsification using a mixture of hydrophobic silica and surfactants

Incubation time [hours]	Yield	FFA [wt.-%]
2	86.7 %	0.36
4	84.6 %	0.38
6	88.4 %	0.41
24	89.0 %	0.70

[0174] As can be seen, and in accordance with the results as disclosed in table 1, an efficient demulsification takes already place with short incubation times, but better yields are achieved with incubation times of more than 5 hours. - The advantage of relatively short incubation times is the very low amount of free fatty acids (FFA) in the final product.

Example 5: Demulsification at different temperatures

[0175] To four samples of 1000 g of the lysed and concentrated fermentation broth as obtained according to example 1 were added 5 g of a demulsifier mixture containing 47.5 wt.-% Tween 80, 47.5 wt.-% Span 80 and 5 wt.-% Sipernat® D17 (Evonik Industries, Germany), respectively, corresponding to a final amount of hydrophobic silica in the suspensions of 0.025 wt.-%.

[0176] The samples such obtained were heated in agitated vessels for 24 hours at different temperatures of 30, 50, 70 and 90°C, respectively. Samples of 100 ml were taken after 24

hours, respectively, and oil was subsequently separated from the aqueous phase by centrifugation.

[0177] The results are disclosed in the following table.

Table 4: Demulsification at different temperatures

Incubation temperature [°C]	Yield	FFA [wt.-%]
30	77.8 %	0.37
50	72.0 %	0.38
70	85.0 %	0.40
90	89.0 %	0.70

[0178] As can be seen, demulsification takes place already at very low temperatures, but good yields are obtained at temperatures above 50°C. - Like for low incubation times, lower FFA values are also obtained at incubation at lower temperatures.

Example 6: Variation of the amount and nature of surfactants in the demulsifier mixture

[0179] To samples of 1000 g of the lysed and concentrated fermentation broth as obtained according to example 1 were added different amounts of a demulsifier mixture containing the hydrophobic silica Sipernat® D17 (Evonik Industries, Germany) and optionally different amounts of surfactants.

[0180] The samples such obtained were heated in agitated vessels at 90°C. Samples of 100 ml were taken after 24 hours, respectively, and oil was subsequently separated from the aqueous phase by centrifugation.

[0181] The different compositions of the demulsifier mixture and amounts as added are disclosed in the following table, as well as the yields of oil as obtained:

Table 5: Composition and employed amounts of demulsifier mixtures

Comp. No.	Surfactant 1 [wt.-%]	Surfactant 2 [wt.-%]	Hydrophobic silica [wt.-%]
1	--	--	100 Sipernat D17
2	45.5 Tween 80	45.5 Span 80	9.0 Sipernat D17
3	48.4 Tween 80	48.4 Span 80	3.2 Sipernat D17
4	47.5 Tween 80	47.5 Span 80	5.0 Sipernat D17
5	71.4 Tween 80	23.8 Span 80	4.8 Sipernat D17
6	23.8 Tween 80	71.4 Span 80	4.8 Sipernat D17
7	47.6 Brij 98	47.6 Brij 92	4.8 Sipernat D17

Table 6: Influence of surfactants on the oil yield

Comp. No.	Amount added [g]	Amount of hydrophobic silica in aq. suspension	Yield	FFA [wt.-%]
1	0.24	0.024 wt.-%	84.2 %	0.70
2	2.5	0.023 wt.-%	86.4 %	0.75
3	7.5	0.024 wt.-%	90.2 %	0.75
4	5	0.025 wt.-%	89.0 %	0.70
5	5	0.024 wt.-%	88.6 %	0.70
6	5	0.024 wt.-%	86.5 %	0.75
7	5	0.024 wt.-%	87.7 %	0.60

[0182] As can be seen, efficient demulsification does take place with different surfactants and even without using surfactants. But surfactants obviously have a positive effect on the yield.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patentkrav

1. Fremgangsmåde til isolering af lipider fra en lipid-indeholdende biomasse, som omfatter følgende trin:

a) tilvejebringelse af en biomassesuspension, hvor cellerne i biomassen indeholder i gennemsnit mindst 10 vægt-% lipider;

b) tilsætning af hydrofob silica til suspensionen;

c) opvarmning af den således opnåede suspension til en temperatur på mindst 50 °C og inkubering af suspensionen i mindst 20 minutter;

d) separering af den olieholdige lette fase fra vandet, saltene, celledebris og den resterende olieholdige vandfase.

2. Fremgangsmåde ifølge krav 1, hvor hydrofob silica tilsættes til suspensionen til justering af en slutkoncentration på mellem 0,005 og 0,25 vægt-%, især mellem 0,008 og 0,20 vægt-%, fortrinsvis 0,012 til 0,15 vægt-%, mere fortrinsvis 0,015 til 0,10 vægt-% hydrofob silica.

3. Fremgangsmåde ifølge krav 1 eller 2, hvor der foruden silica tilsættes mindst ét overfladeaktivt stof, fortrinsvis mindst ét ikke-ionisk overfladeaktivt stof til suspensionen, fortrinsvis i en mængde til justering af en slutkoncentration på mellem 0,1 og 5,0 vægt-%, mere fortrinsvis mellem 0,15 og 4,0 vægt-%, især mellem 0,25 og 3,0 vægt-% eller mellem 0,3 og 2,0 vægt-%.

4. Fremgangsmåde ifølge et hvilket som helst af ovennævnte krav, hvor biomassesuspensionen ifølge trin (a) har et totalt tørstofindhold på mellem 20 og 60 vægt-%.

5. Fremgangsmåde ifølge et hvilket som helst af ovennævnte krav, hvor suspensionen opvarmes i trin (c) til en temperatur på 50 til 100 °C, fortrinsvis 65 til 95 °C.

6. Fremgangsmåde ifølge et hvilket som helst af ovennævnte krav, hvor suspensionen inkuberes i trin (c) i 20 minutter til 30 timer, fortrinsvis i 1 time til 24 timer.

7. Fremgangsmåde ifølge et hvilket som helst af ovennævnte krav, der som et yderligere trin omfatter lysering af cellerne i biomassen, hvor lyseringen af cellerne fortrinsvis udføres før tilsætning af hydrofob silica.

8. Fremgangsmåde ifølge et hvilket som helst af ovennævnte krav, hvor biomassesuspensionen er en fermenteringssuppe, og cellerne fortrinsvis er celler fra rækken Stramanopiles, især fra familien Thraustochytrids, fortrinsvis fra slægten Schizochytrium.

9. Oile, som omfatter hydrofob silica i en mængde på 5 ppm (vægt/vægt) til 10 vægt-%, fortrinsvis 10 ppm (vægt/vægt) til 5 vægt-%, især 0,01 vægt-% til 4 vægt-%, 0,1 vægt-% til 3 vægt-% eller 0,5 vægt-% til 2,5 vægt-%, hvor olien fortrinsvis omfatter PUFA'er i en mængde på mindst 10 vægt-%, fortrinsvis mindst 20 vægt-%, især 25 til 70 vægt-%, 30 til 65 vægt-% eller 45 til 60 vægt-%.

10. olie ifølge krav 9, hvor olien omfatter fedtsyreestere, især TAG'er, i en mængde på mindst 50 vægt-%, fortrinsvis mindst 70 eller 80 vægt-%, især i en mængde på mindst 85 eller 90 vægt-%, der er kendetegnet ved at omfatte frie fedtsyrer (FFA) i en mængde på mindre end 1 vægt-%, fortrinsvis mindre end 0,8 vægt-%, mere fortrinsvis mindre end 0,5 vægt-%.

11. PUFA-indeholdende aflipideret biomasse, som omfatter 10 til 25 vægt-%, især 15 til 20 vægt-% aske og mindre end 2 vægt-%, mere fortrinsvis mindre end 0,5 vægt-%, især mindre end 0,1 vægt-% af et ikke-polært organisk opløsningsmiddel og mindre end 2 vægt-%, mere fortrinsvis mindre end 0,5 vægt-%, især mindre end 0,1 vægt-% chlorid, hvor biomassen indeholder celler og/eller celledebris fra rækken Stramanopiles, især fra familien Thraustochytrids, fortrinsvis fra slægten Schizochytrium.

12. Biomasse ifølge krav 11, som omfatter en blanding af DHA og EPA, fortrinsvis i et forhold på ca. 3:2 til ca. 4:1, hvor indholdet af DHA er mindst 10 vægt-% (i forhold til den samlede

mængde indeholdt lipid).

13. PUFA-indeholdende vandig suspension, som indeholder en biomasse, der er kendetegnet ved et indhold af aske på mindre end 15 vægt-%, fortrinsvis 6 til 10 vægt-% og et indhold af ikke-polære organiske opløsningsmidler på mindre end 1 vægt-%, fortrinsvis mindre end 0,1 vægt-%, og et indhold af chlorid på mindre end 1 vægt-%, fortrinsvis mindre end 0,1 vægt-%, hvor biomassen indeholder celler og/eller celledebris fra rækken Stramanopiles, især fra familien Thraustochytrids, mere fortrinsvis fra slægten Schizochytrium, og hvor den vandige suspension er kendetegnet ved et samlet tørstofindhold på 20 til 60 vægt-%.

14. Fremgangsmåde til fodring af dyr, især kødkvæg, hvor en olie ifølge krav 9 eller 10 eller en biomasse ifølge et hvilket som helst af kravene 11 eller 12 og/eller en vandig suspension ifølge krav 13 tilvejebringes til dyr, fortrinsvis efter blanding af olien, den partikelformige biomasse og/eller den vandige suspension med yderligere foderstofbestanddele.