

CLAIMS

1. A process for producing extracted lipid, comprising extracting lipid from microbial cells which comprise triacylglycerol (TAG) molecules, each TAG molecule
5 having a fatty acid esterified at each of an sn-1 position, an sn-2 position and an sn-3 position, wherein at least some of the TAG molecules comprise a SCFA esterified at the sn-3 position, so as to thereby produce the extracted lipid.
2. The process of claim 1, wherein the esterified SCFA in the TAG molecules are
10 one or more or all of butyric acid (C4:0), caproic acid (C6:0) and caprylic acid (C8:0), preferably comprising C4:0, or C4:0 and C6:0, more preferably C4:0, C6:0 and C8:0.
3. The process of claim 1 or claim 2, wherein the extracted lipid is characterised by one or more or all of:
15 (i) the lipid comprises TAG molecules which comprise a SCFA esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise a SCFA esterified at the sn-2 position to the number of TAG molecules which comprise a SCFA esterified at their sn-3 position (SCFA ratio sn-2:sn-3) in the extracted lipid is less than about 0.50, less than about 0.30, less than about 0.20, less than about 0.10,
20 less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
(ii) the lipid comprises TAG molecules which comprise C4:0 esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise C4:0 esterified at the sn-2 position to the number of TAG molecules which comprise C4:0 esterified at their sn-3 position (sn-2:sn-3 ratio for C4:0) in the extracted lipid is less
25 than about 0.50, less than about 0.30, less than about 0.20, less than about 0.10, less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
(iii) the lipid comprises TAG molecules which comprise C6:0 esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise C6:0 esterified at the sn-2 position to the number of TAG molecules which comprise C6:0
30 esterified at their sn-3 position (sn-2:sn-3 ratio for C6:0) in the extracted lipid is less than about 0.50, less than about 0.30, less than about 0.20, less than about 0.10, less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
(iv) the sn-2:sn-3 ratio for SCFA is more than about 0.005, more than about 0.01, between about 0.005 and about 0.10, between about 0.01 and about 0.10, between
35 about 0.005 and about 0.05 or between about 0.01 and about 0.05,

- (v) the sn-2:sn-3 ratio for C4:0 is more than about 0.005, more than about 0.01, between about 0.005 and about 0.10, between about 0.01 and about 0.10, between about 0.005 and about 0.05 or between about 0.01 and about 0.05,
- (vi) the sn-2:sn-3 ratio for C6:0 is more than about 0.005, more than about 0.01, 5 between about 0.005 and about 0.10, between about 0.01 and about 0.10, between about 0.005 and about 0.05 or between about 0.01 and about 0.05,
- (vii) the SCFA comprises C4:0 and the sn-2:sn-3 ratio for C4:0 is more than about 0.01 or more than about 0.02,
- (viii) more of the TAG molecules in the extracted lipid have only one SCFA esterified 10 in the TAG molecule than two SCFA esterified in the TAG molecule,
- (ix) at least 1% by weight or by mol%, or both, of the total fatty acid content of the extracted lipid is SCFA, preferably at least 1% C4:0, or at least 1% for the sum of C4:0 and C6:0,
- (x) at least 10%, at least 15%, at least 20%, at least 25% or at least 30% by weight 15 of the total fatty acid content of the extracted lipid is C16:1, preferably to a maximum of 50%, and
- (xi) the TAG molecules of the extracted lipid comprise more C16:1 esterified at the sn-2 position than any other fatty acid esterified at the sn-2 position, preferably wherein at least 30% or at least 40% of the fatty acids esterified at the sn-2 position of the TAG 20 molecules are C16:1.
4. The process of any one of claims 1 to 3, wherein the microbial cells comprise fungal cells, bacterial cells or algal cells, or any mixture thereof, preferably yeast cells, more preferably *Saccharomyces cerevisiae* cells. 25
5. The process of any one of claims 1 to 4, wherein the cells comprise one or more exogenous polynucleotide(s) encoding a fatty acid acyltransferase or more than one fatty acid acyltransferase, wherein at least one acyltransferase has activity on a SCFA-CoA molecule as a substrate, preferably at least butyryl-CoA as a substrate, and 30 wherein each exogenous polynucleotide is operably linked to one or more promoters that are capable of directing expression of said polynucleotide in the cells.
6. The process of claim 5, wherein at least one of the fatty acid acyltransferases is one or more of:
- 35 (i) a mammalian acyltransferase or a variant thereof, preferably a ruminant acyltransferase or a variant thereof,

- (ii) a diacylglycerol O-acyltransferase (DGAT), preferably a DGAT1,
 - (iii) a 1,2-diacyl-sn-glycerol:acetyl-CoA acetyltransferase (DAGAT),
 - (iv) a mammalian DGAT or a variant thereof, preferably a ruminant DGAT or a variant thereof, and
- 5 (v) a DGAT comprising amino acids whose sequence is set forth as any one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, or an amino acid sequence which is at least 30% identical along the full length of any one or more of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29.
- 10 7. The process of any one of claims 1 to 6, wherein step of extracting the lipid comprises exposing the cells to an organic solvent, pressing the cells or treating the cells with microwave irradiation, ultrasonication, high-speed homogenization, high-pressure homogenization, bead beating, autoclaving, thermolysis or any combination thereof.
- 15 8. The process of any one of claims 1 to 7, wherein the method further comprises culturing the cells, preferably in a medium comprising butyrate (C4:0), such as at a concentration in the range of about 1 to about 20 g/L in the medium, and/or further comprises modifying or purifying the lipid after extraction.
- 20 9. Extracted microbial lipid comprising triacylglycerol (TAG) molecules, each TAG molecule having a fatty acid esterified at each of an sn-1 position, an sn-2 position and an sn-3 position, wherein at least some of the TAG molecules comprise a short chain fatty acid (SCFA) esterified at the sn-3 position of the TAG molecule.
- 25 10. The lipid of claim 9, wherein the SCFAs esterified at the sn-3 position of the TAG molecules are one or more or all of C4:0, C6:0 and C8:0, preferably C4:0 and C6:0, more preferably C4:0, C6:0 and C8:0.
- 30 11. The lipid of claim 9 or claim 10, which is characterised by one or more or all of:
- (i) the extracted lipid comprises TAG molecules which comprise a SCFA esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise a SCFA esterified at the sn-2 position to the number of TAG molecules which comprise a SCFA esterified at their sn-3 position (SCFA ratio sn-2:sn-3) in the
- 35 extracted lipid is less than about 0.50, less than about 0.30, less than about 0.20, less

- than about 0.10, less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
- (ii) the extracted lipid comprises TAG molecules which comprise C4:0 esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise C4:0 esterified at the sn-2 position to the number of TAG molecules which comprise C4:0 esterified at their sn-3 position (sn-2:sn-3 ratio for C4:0) in the extracted lipid is less than about 0.50, less than about 0.30, less than about 0.20, less than about 0.10, less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
- (iii) the extracted lipid comprises TAG molecules which comprise C6:0 esterified at their sn-2 position, wherein the ratio of the number of TAG molecules which comprise C6:0 esterified at the sn-2 position to the number of TAG molecules which comprise C6:0 esterified at their sn-3 position (sn-2:sn-3 ratio for C6:0) in the extracted lipid is less than about 0.50, less than about 0.30, less than about 0.20, less than about 0.10, less than about 0.05, less than about 0.04, less than about 0.03 or less than about 0.02,
- (iv) the sn-2:sn-3 ratio for SCFA is more than about 0.005, more than about 0.01, between about 0.005 and about 0.10, between about 0.01 and about 0.10, between about 0.005 and about 0.05 or between about 0.01 and about 0.05,
- (v) the sn-2:sn-3 ratio for C4:0 is more than about 0.005, more than about 0.01, between about 0.005 and about 0.10, between about 0.01 and about 0.10, between about 0.005 and about 0.05 or between about 0.01 and about 0.05,
- (vi) the sn-2:sn-3 ratio for C6:0 is more than about 0.005, more than about 0.01, between about 0.005 and about 0.10, between about 0.01 and about 0.10, between about 0.005 and about 0.05 or between about 0.01 and about 0.05,
- (vii) the SCFA comprises C4:0 and the SCFA ratio sn-2:sn-3 is more than about 0.01 or more than about 0.02,
- (viii) more of the TAG molecules in the extracted lipid have only one SCFA esterified to the TAG molecule than two SCFA esterified to the TAG molecule,
- (ix) at least 1% by weight or by mol%, or both, of the total fatty acid content of the extracted lipid is SCFA, preferably C4:0, or the sum of C4:0 and C6:0, or the sum of C4:0, C6:0 and C8:0,
- (x) at least 10%, at least 15%, at least 20%, at least 25% or at least 30% by weight of the total fatty acid content of the extracted lipid is C16:1, preferably to a maximum of 50%, and
- (xi) the TAG molecules of the extracted lipid comprise more C16:1 esterified at the sn-2 position than any other fatty acid esterified at the sn-2 position, preferably wherein

at least 30% or at least 40% of the fatty acids esterified at the sn-2 position of TAG are C16:1.

12. A microbial cell comprising

5 (a) triacylglycerol (TAG) molecules, each TAG molecule having a fatty acid esterified at each of an sn-1 position, an sn-2 position and an sn-3 position, wherein at least some of the TAG molecules comprise a short chain fatty acid (SCFA) esterified at the sn-3 position of the TAG molecule, and/or

(b) one or more exogenous polynucleotide(s) encoding a fatty acid
10 acyltransferase or more than one fatty acid acyltransferase, wherein at least one acyltransferase has activity on SCFA-CoA molecules as a substrate, preferably at least butyryl-CoA as a substrate or at least butyryl-CoA and caproyl-CoA as substrates, and wherein each exogenous polynucleotide is operably linked to one or more promoters that are capable of directing expression of said polynucleotide in the cells.

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13. The cell of claim 12, wherein the TAG molecules or the fatty acid acyltransferase has one or more of the features defined in claim 5 or claim 6.

14. A process for culturing microbial cells, the process comprising

20 (a) obtaining a microbial cell which produces TAG molecules, each TAG molecule having a fatty acid esterified at each of an sn-1 position, an sn-2 position and an sn-3 position, wherein at least some of the TAG molecules comprise a short chain fatty acid (SCFA) esterified at the sn-3 position of the TAG molecule, and/or

(b) obtaining a microbial cell which comprises one or more exogenous
25 polynucleotide(s) encoding a fatty acid acyltransferase or more than one fatty acid acyltransferase, wherein at least one acyltransferase has activity on SCFA-CoA molecules as a substrate, preferably at least butyryl-CoA as a substrate or at least butyryl-CoA and caproyl-CoA as substrates, and wherein each exogenous polynucleotide is operably linked to one or more promoters that are capable of directing
30 expression of said polynucleotide in the cells, and

(c) increasing the number of the cells by culturing the cells in a suitable medium.

15. The process of claim 14, wherein the medium comprises butyrate (C4:0).

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16. A process for producing a microbial cell which produces TAG molecules, the process comprising a step of introducing one or more exogenous polynucleotides which encodes one or more fatty acid acyltransferases as defined in claim 5 or 6.
- 5 17. A process for selecting or identifying a microbial cell of claim 12 or claim 13, comprising steps of
- (i) analysing lipid from microbial cells comprising one or more exogenous polynucleotides encoding a fatty acid acyltransferase as defined in claim 5 or claim 6, and
- 10 (ii) selecting or identifying a microbial cell from step i) which is a microbial cell of claim 12 or claim 13.
18. A composition comprising one or both of the lipid of any one of claims 9 to 11 or the microbial cell of claim 12 or claim 13, preferably with another food or beverage
- 15 ingredient.
19. A food, feedstuff or beverage comprising one or more of extracted lipid of any one of claims 9 to 11, the microbial cell of claim 12 or claim 13, or the composition of claim 18, and least one other food, feed or beverage ingredient.
- 20 20. The food, feedstuff or beverage of claim 19 which has no components derived from an animal.
21. A method of producing a food, feedstuff or beverage ingredient, or a food,
- 25 feedstuff or beverage, the method comprising mixing one or more of extracted lipid of any one of claims 9 to 11, the microbial cell of claim 12 or claim 13, or the composition of claim 18, with at least one other food or feedstuff ingredient, and/or processing the extracted lipid of any one of claims 9 to 11, the microbial cell of claim 12 or claim 13, or the composition of claim 18.
- 30 22. Use of one or more of extracted lipid of any one of claims 9 to 11, the microbial cell of claim 12 or claim 13, or the composition of claim 18 to produce a food, feedstuff or beverage ingredient, or a food, feedstuff or beverage.