IN THE UNITED STATES PATENT & TRADEMARK RECEIVING OFFICE
PCT PATENT APPLICATION

MICROBIALLY PRODUCED PALM OIL SUBSTITUTES

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of priority to U.S. Provisional Application No. 62/972,299, filed on February 10, 2020, and to U.S. Provisional Application No. 63/061,521, filed on August 5, 2020, the contents of each of which are herein incorporated by reference in their entireties.

FIELD OF THE DISCLOSURE

[2] The present disclosure relates to environmentally friendly and sustainable alternatives to plant-derived palm oil. The palm oil alternatives are produced by oleaginous microorganism and share one or more features with plant-derived palm oils. These alternatives may also be fractionated, treated, and/or derivatized based on their intended use.

BACKGROUND

[3] Palm oil is currently the most widely produced vegetable oil on the planet, as it finds uses in the manufacture of a large variety of products. It is widely used in food, as a biofuel precursor, and in soaps and cosmetics. The global demand for palm oil is approximately 57 million tons and is steadily increasing. However, the high demand for palm oil has resulted in environmentally detrimental practices related to the expansion of plantations devoted to palm oil-producing plants. Palm oil production is a leading contributor to tropical deforestation, resulting in habitat destruction, increased carbon dioxide emissions, and local smog clouds across South East Asia.

[4] Thus, there is an urgent need for palm oil alternatives that do not rely upon utilization of oil palms and incur the associated negative environmental costs.

SUMMARY

[5] In one example, the present disclosure provides a refined, bleached, and/or deodorized (RBD) microbial oil composition produced by an oleaginous yeast.

[5a] In one aspect, the present disclosure refers to a refined, bleached, and/or deodorized (RBD) microbial oil composition produced by an oleaginous yeast.

[6] In one example, the present disclosure provides a refined, bleached, and/or deodorized (RBD) microbial oil composition produced by an oleaginous yeast, wherein the composition comprises ergosterol and does not comprise campesterol, β-sitosterol, or stigmasterol.

[7] In one example, the present disclosure provides a refined and/or deodorized microbial oil composition produced by an oleaginous yeast, wherein the composition comprises at least one pigment selected from the group consisting of carotene, torulene and torulorhodin and does not comprise chlorophyll.

[8] In some examples, the composition is bleached, thereby producing an RBD microbial oil composition, but wherein a measurable amount of the pigment remains.

[9] In one example, the present disclosure provides a refined, bleached, and/or deodorized (RBD) microbial oil composition produced by an oleaginous yeast, wherein the composition is fractionable into two fractions, wherein the two fractions are microbial olein and microbial stearin, wherein each fraction comprises at least 10% of the composition's original mass, and wherein the iodine value (IV) of the fractions differs by at least 10.

[10] In one example, the present disclosure provides a microbial oil composition produced by an oleaginous yeast, wherein the composition comprises the following amounts of fatty acids relative to the total fatty acids: at least about 30% w/w saturated fatty acids with chain lengths between 16 and 18 carbons long; at least about 30% w/w unsaturated fatty acids with 18 carbon chain lengths; and less than about 30% w/w total polyunsaturated fatty acids.

[11] In one example, the present disclosure provides a refined, bleached, and/or deodorized (RBD) microbial oil composition produced by an oleaginous yeast, wherein the composition has one or more characteristics similar to plant-derived palm oil selected from the group consisting of: apparent density, refractive index, saponification value, unsaponifiable matter, iodine value, slip melting point, fatty acid composition, triglyceride content, overall saturation level, and level of mono- and poly-unsaturated fatty acids.

[12] In one example, the present disclosure provides a microbial oil composition produced by an oleaginous yeast, comprising: at least about 30% w/w saturated fatty acids with chain lengths between 16 and 18 carbons long; at least about 30% w/w unsaturated fatty acids with 18 carbon chain lengths; less than about 30% w/w total polyunsaturated fatty acids; at least about 50 ppm

ergosterol; wherein the composition does not contain a phytosterol or chlorophyll, and wherein the composition has one or more characteristics similar to plant-derived palm oil selected from the group consisting of iodine value, triglyceride content, slip melting point, oxidative stability, and overall saturation level.

[13] In some examples, the composition comprises 10-45% C16 saturated fatty acid.

[14] In some examples, the composition comprises 10-70% C18 unsaturated fatty acid.

[15] In some examples, the composition comprises 3-30% C18 saturated fatty acid.

[16] In some examples, the composition comprises a saponification value similar to that of plant-derived palm oil.

[17] In some examples, the composition comprises a saponification value of 150-210.

[18] In some examples, the composition comprises an iodine value similar to that of plant-derived palm oil.

[19] In some examples, the composition comprises an iodine value of 50-65.

[20] In some examples, the composition comprises a slip melting point similar to that of plant-derived palm oil.

[21] In some examples, the composition comprises a slip melting point of 30°C-40°C.

[22] In some examples, the composition comprises a saturated fatty acid composition similar to that of plant-derived palm oil.

[23] In some examples, the composition comprises a saturated fatty acid composition of at least 30%.

[24] In some examples, the composition comprises a saturated fatty acid composition of at most 70%.

[25] In some examples, the composition comprises an unsaturated fatty acid composition similar to that of plant-derived palm oil.

[26] In some examples, the composition comprises an unsaturated fatty acid composition of at least 30%.

[27] In some examples, the composition comprises an unsaturated fatty acid composition of at most 70%.

[28] In some examples, the composition comprises a mono- and poly-unsaturated fatty acid composition similar to that of plant-derived palm oil.

[29] In some examples, the composition comprises 30-50% mono-unsaturated fatty acids as a percentage of overall fatty acids.

[30] In some examples, the composition comprises 5-25% poly-unsaturated fatty acids as a percentage of overall fatty acids.

[31] In some examples, the composition comprises a triglyceride content similar to that of plant-derived palm oil.

[32] In some examples, the composition comprises a triglyceride content of 90-98% as a percentage of overall glycerides.

[33] In some examples, the composition comprises less than 100 ppm of, comprises less than 50 ppm of, or does not comprise a sterol selected from a phytosterol, cholesterol, or a prototheca sterol.

[34] In some examples, the composition comprises less than 100 ppm of, comprises less than 50 ppm of, or does not comprise a phytosterol.

[35] In some examples, the composition comprises less than 100 ppm of, comprises less than 50 ppm of, or does not comprise a phytosterol selected from the group consisting of campesterol, β-sitosterol, stigmasterol.

[36] In some examples, the composition comprises less than 100 ppm of, comprises less than 50 ppm of, or does not comprise cholesterol.

[37] In some examples, the composition comprises less than 100 ppm of, comprises less than 50 ppm of, or does not comprise prototheca sterol.

[38] In some examples, the composition comprises ergosterol, comprises at least 50 ppm ergosterol, or comprises at least 100 ppm ergosterol.

[39] In some examples, the composition comprises an ergosterol content of at least 60% w/w as a percentage of overall sterols.

[40] In some examples, the composition does not comprise a pigment.

[41] In some examples, the composition does not comprise chlorophyll.

[42] In some examples, the composition comprises a pigment selected from the group consisting of carotene, torulene and torulorhodin.

[43] In some examples, the composition comprises each of carotene, torulene and torulorhodin.

[44] In some examples, the composition comprises at least 10 ppm, at least 50 ppm, or at least 100 ppm carotene.

[45] In some examples, the composition comprises carotene, and wherein the carotene is β-carotene and/or a derivative thereof.

[46] In some examples, the composition comprises at least 10 ppm, at least 50 ppm, or at least 100 ppm torulene and/or a derivative thereof.

[47] In some examples, the composition comprises at least 10 ppm, at least 50 ppm, or at least 100 ppm torulorhodin and/or a derivative thereof.

[48] In some examples, the oleaginous yeast is a recombinant yeast.

[49] In some examples, the oleaginous yeast is of the genus *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Metschnikowia, Cryptococcus, Trichosporon,* or *Lipomyces*.

[50] In some examples, the oleaginous yeast is of the genus *Rhodosporidium*.

[51] In some examples, the oleaginous yeast is of the species *Rhodosporidium toruloides*.

[52] In some examples, the composition is fractionable.

[53] In some examples, the composition may be fractionated into microbial olein and microbial stearin.

[54] In some examples, the composition may be fractionated into microbial olein and microbial stearin, and wherein each fraction comprises at least 10% of the composition's starting mass.

[55] In some examples, the composition may be fractionated into microbial olein and microbial stearin, and wherein the iodine value (IV) of the fractions differs by at least 10.

[56] In some examples, the composition may be fractionated into microbial olein and microbial stearin, and wherein the IV of the fractions differs by at least 20.

[57] In some examples, the composition may be fractionated into microbial olein and microbial stearin, and wherein the IV of the fractions differs by at least 30.

[58] In one example, the present disclosure provides a microbial oil composition produced by an oleaginous yeast, wherein the composition comprises: less than 10% w/w palmitic-palmitic-palmitic triglycerides; greater than 15% w/w palmitic-palmitic-oleic triglycerides; and greater than 15% w/w oleic-oleic-palmitic triglycerides.

[59] In some examples, said palmitic-palmitic-palmitic triglyceride content is between about 0.8% and 1.3% w/w.

[60] In some examples, said palmitic-palmitic-oleic triglyceride content is between about 16.9% and 28.2% w/w.

[61] In some examples, said oleic-oleic-palmitic triglyceride content is between about 15.7% and 26.0% w/w.

[62] In some examples, the composition further comprises a stearic-stearic-oleic triglyceride content of less than 10% w/w and a stearic-oleic-oleic triglyceride content of less than 10% w/w.

[63] In some examples, said stearic-stearic-oleic triglyceride content is between about 1.2% and 1.9% w/w.

[64] In some examples, said stearic-oleic-oleic triglyceride content is between about 3.2% and 5.4% w/w.

[65] In one example, the present disclosure provides a microbial oil composition produced by an oleaginous yeast, wherein the composition comprises triglycerides, and wherein greater than 40% of said triglycerides have one unsaturated sidechain.

[66] In some examples, greater than 30% of said triglycerides have two unsaturated sidechains.

[67] In some examples, between 10% and 15% of palmitic and/or stearic fatty acids are located at the sn-2 position of triglyceride molecules.

[68] In one example, the present disclosure provides a microbial oil composition produced by an oleaginous yeast, wherein the composition comprises the following amounts of fatty acids relative to the total fatty acids: between about 7.0% and 35% stearic acid; between about 10% and 50% oleic acid; and between about 8% and 20% linoleic acid.

[69] In one example, the present disclosure provides a method of producing a microbial oil composition according to any one of the foregoing examples, the method comprising the steps of: providing an oleaginous yeast and a carbon source, and culturing said oleaginous yeast, thereby producing said microbial oil.

[69a] In one aspect, the present disclosure refers to a method of producing the microbial oil composition disclosed herein, the method comprising the steps of: a) providing an oleaginous yeast and a carbon source, and b) culturing said oleaginous yeast, thereby producing said microbial oil composition.

[70] In some examples, the methods and compositions recited in International Patent Application No. PCT/US2021/015302, incorporated by reference herein, are employed in the compositions and methods of the disclosure. In some examples, the feedstocks of International Patent Application No. PCT/US2021/015302 are utilized in the compositions and methods of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[71] The accompanying figures, which are incorporated herein and form a part of the specification, illustrate some, but not the only or exclusive, example embodiments and/or features. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than limiting.

[72] **FIG. 1A** shows a chromatogram of the fatty acid composition analysis of exemplary crude microbial oil; **FIG. 1B** shows a chromatogram of the fatty acid composition analysis of exemplary crude palm oil; **FIG. 1C** shows a chromatogram of the fatty acid composition analysis of exemplary crude hybrid palm oil; and **FIG. 1D** shows a bar graph of representative fatty acid compositions of microbial oil and palm oil.

[73] **FIG. 2A** shows a chromatogram of the triglyceride composition analysis of exemplary crude microbial oil; **FIG. 2B** shows a chromatogram of the triglyceride composition analysis of exemplary crude palm oil; and **FIG. 2C** shows a chromatogram of the triglyceride composition analysis of exemplary crude hybrid palm oil.