FOOD COMPOSITIONS COMPRISING TAILORED OILS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. 119(e) of US Provisional Patent Application No. 61/349,774, filed May 28, 2010, US Provisional Patent Application No. 61/374,992, filed August 18, 2010, US Provisional Patent Application No. 61/414,393, filed November 16, 2010, and US Provisional Patent Application No. 61/428,192, filed December 29, 2010. Each of these applications is incorporated herein by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING

[0002] This application includes a sequence listing as shown in pages 1-195, appended hereto.

FIELD OF THE INVENTION

[0003] The present invention relates to the production of food compositions, oils, fuels, and oleochemicals made from microorganisms. In particular, the disclosure relates to oil-bearing microalgae, methods of cultivating them for the production of biomass and useful compounds, including lipids, fatty acid esters, fatty acids, aldehydes, alcohols, and alkanes, and methods and reagents for genetically altering them to improve production efficiency and alter the type and composition of the oils produced by them.

BACKGROUND OF THE INVENTION

[0004] As the human population continues to increase, there's a growing need for additional food sources, particularly food sources that are inexpensive to produce but nutritious. Moreover, the current reliance on meat as the staple of many diets, at least in the most developed countries, contributes significantly to the release of greenhouse gases, and there's a need for new foodstuffs that are equally tasty and nutritious yet less harmful to the environment to produce. There remains a need for methods to produce foodstuffs from microorganism, including algae, cheaply and efficiently, at large scale, particularly foodstuffs that are tasty and nutritious. The present invention meets these and other needs

SUMMARY OF THE INVENTION

[0005] The present invention provides oleaginous microbial cells, preferably microalgal cells, having distinct lipid profiles, and includes recombinant cells expressing exogenous genes encoding proteins such as fatty acyl-ACP thioesterases. ~~The present invention a~~Also described are~~provides~~ methods of making lipids and oil-based products, including fuels such as biodiesel, renewable diesel and jet fuel, from such cells.

[0005a] In a first aspect, the present invention refers to a microbial oil obtained from a recombinant microalga, wherein the fatty acid profile of the oil comprises at least 40% saturated fatty acid having C10, C12 and C14, and the oil further comprises sterols of the recombinant microalga, wherein the recombinant microalga has been genetically engineered to express at least one lipid pathway enzyme at an altered level compared to a non-engineered microalga, the lipid pathway enzyme being selected from the group consisting of a desaturase, a ketoacyl synthase, and an acyl-ACP thioesterase.

[0005b] In a second aspect, the present invention refers to a blended composition comprising a microbial oil of the present invention and an oil from soy, rapeseed, canola, palm, palm kernel, coconut, corn, waste vegetable, Chinese tallow, olive, sunflower, cottonseed, chicken fat, beef tallow, porcine tallow, microalgae, macroalgae, microbes, Cuphea, flax, peanut, choice white grease, lard, Camelina sativa, mustard seed, cashew nut, oats, lupine, kenaf, calendula, help, coffee, linseed (flax), hazelnut, euphorbia, pumpkin seed, coriander, camellia, sesame, safflower, rice, tung tree, cocoa, copra, opium poppy, castor beans, pecan, jojoba, macadamia, Brazil nuts, avocado, petroleum, or a distillate fraction of any of the preceding oils.

[0006] In a first exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 1% or at least 5%, preferably at least 3%, C8:0. In some cases, the lipid profile is at least 10% or at least 15%, preferably at least 12%, C8:0. In some examplembodiments, the cell is a recombinant cell. In some cases, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C8. In some examplembodiments, the exogenous gene encodes a *Cuphea palustris* acyl-ACP thioesterase. In some cases, the cell is a *Prototheca* cell. In some cases, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0007] In a second exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 4% C10:0. In some cases, the lipid profile is at least 20%, at least 25% or at least 30%, preferably at least 24%, C10:0. In some cases, the ratio of C10:0 to C12:0 is at least 6:1. In some examplembodiments, the cell is a recombinant cell. In some cases, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C10. In some examplembodiments, the exogenous gene encodes an acyl-ACP thioesterase protein from a species selected from the group consisting of *Cuphea hookeriana* and *Ulmus americana*. In some cases, the cell is a *Prototheca* cell. In some examplembodiments, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0008] In a third exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 10% or at least 15%, prefereably at least 13%, C12:0. In some cases, the lipid profile is at least 30%, at least 35% or at least 40%, preferably at least 34%, C12:0. In some cases, the ratio of C12 to C14 is at least 5:1. In some cases, the cell is a recombinant cell. In some examplembodiments, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C12. In some cases, the recombinant cell comprises at least two exogenous genes encoding acyl-ACP thioesterase proteins from *Umbellularia californica* and *Cinnamomum camphora* that have hydrolysis activity towards fatty acyl-ACP substrates of chain length C12. In some embodiments, the cell is a *Prototheca* cell.

[0009] In a fourth exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 5% or at least 15%, preferably at least 10%, C14:0. In some cases, the lipid profile is at least 40%, at least 45%,

or at least 50%, preferably at least 43%, C14:0. In some cases, the ratio of C14:0 to C12:0 is at least 7:1. In some cases, the cell is a recombinant cell. In some examplembodiments, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C14. In some examplembodiments, the acyl-ACP thioesterase protein is from a species selected from the group consisting of *Cinnamomum camphora* and *Ulmus americana*. In some cases, the cell is a *Prototheca* cell. In some examplembodiments, the cell is of a microalgal genus or species selected from microalgae identified in Table 1.

[0010] In a fifth exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 10% or at least 20%, preferably at least 15%, C16:0. In some cases, the lipid profile is at least 30%, at least 35% or at least 40%, preferably at least 37%, C16:0. In some cases, the cell is a recombinant cell. In some examplembodiments, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain length C16. In some examplembodiments, the recombinant cell comprises at least two exogenous genes encoding acyl-ACP thioesterase proteins from *Umbellularia californica* and *Cinnamomum camphora* that have hydrolysis activity towards fatty acyl-ACP substrates of chain length C16. In some cases, the cell is a *Prototheca* cell.

[0011] In a sixth exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, having a lipid profile that is at least 55% or at least 65%, preferably at least 60%, saturated fatty acids. In some cases the cells,have a lipid profile that is at least 80%, at least 85%, or at least 90%, preferably at least 86%, saturated fatty acids. In some cases, the cell is a recombinant cell. In some examplembodiments, the recombinant cell comprises an exogenous gene encoding an acyl-ACP thioesterase protein that has hydrolysis activity towards fatty acyl-ACP substrates of chain lengths C10-C16. In some examplembodiments, the cell comprises an exogenous gene encoding a ketoacyl synthase protein. In some cases, the cell is a *Prototheca* cell.

[0012] In a seventh exampleaspect, described arethe present invention provides oleaginous microbial cells, preferably microalgal cells, comprising a mutated endogenous desaturase gene, wherein the mutation renders the gene or desaturase inactive. In some cases, the cell has a lipid profile that is at least 40% or at least 50%, preferably at least 45%, saturated fatty acids. In some cases, the cell has a lipid profile that is at least 15%, at least 20% or at least 25%, preferably at least 19%, C18:0. In some examplembodiments, the cell comprises a mutated endogenous desaturase gene that results in at least a 2-fold increase in C18:0 fatty acid, as compared to a

wild-type cell. In some cases, the microalgal cell has a lipid profile that is no more than 1% or no more than 5%, preferably no more than 2%, C18:2. In some example~~embodiments~~, the microalgal cell has a lipid profile that is no more than 5% or no more than 10%, preferably no more than 7%, 18:1.

[0013] In some example~~embodiments~~ of the recombinant cells discussed herein, the cell comprises a mutated endogenous desaturase gene, wherein the mutation renders the gene or desaturase inactive.

[0014] In a eighth example~~aspect~~, described is~~the present invention provides~~ a method of making lipid. In one example~~embodiment~~, the method comprises (a) cultivating a cell as discussed above until the cell is at least 15% or at least 25%, preferably at least 20%, lipid by dry weight, and (b) separating the lipid from water-soluble biomass components.

[0015] In a ninth example~~aspect~~, described is~~the present invention provides~~ another method of making lipid. In one example~~embodiment~~, the method comprises (a) cultivating an oleaginous microbial, preferably a microalgae cell, containing exogenous genes encoding two distinct acyl-ACP thioesterases, wherein the lipid profile of the cell is distinct from (i) the profile of the cell without the exogenous genes and (ii) the profile of the cell with only one of the exogenous genes, and (b) separating the lipid from water-soluble biomass components. In some cases, at least one of the exogenous genes encodes a fatty acyl-ACP thioesterase selected from the group consisting of the thioesterases identified in Table 4.

[0016] In a tenth example~~aspect~~, described is~~the present invention provides~~ a method of making an oil-based product. In one example~~embodiment~~, the method comprises (a) cultivating a cell as discussed above until the cell is at least 5% or at least 15%, preferably at least 10%, lipid by dry weight, (b) separating the lipid from water-soluble biomass components, and (c) subjecting the lipid to at least one chemical reaction selected from the group consisting of: saponification; metathesis; acid hydrolysis; alkaline hydrolysis; enzymatic hydrolysis; catalytic hydrolysis; hot-compressed water hydrolysis; a catalytic hydrolysis reaction wherein the lipid is split into glycerol and fatty acids; an amination reaction to produce fatty nitrogen compounds; an ozonolysis reaction to produce mono- and dibasic-acids; a triglyceride splitting reaction selected from the group consisting of enzymatic splitting and pressure splitting; a condensation reaction that follows a hydrolysis reaction; a hydroprocessing reaction; a hydroprocessing reaction and a deoxygenation reaction or a condensation reaction prior to or simultaneous with the hydroprocessing reaction; a gas removal reaction; a deoxygenation reaction selected from the group consisting of a hydrogenolysis reaction, hydrogenation, a consecutive hydrogenation-hydrogenolysis reaction, a consecutive hydrogenolysis-

hydrogenation reaction, and a combined hydrogenation-hydrogenolysis reaction; a condensation reaction following a deoxygenation reaction; an esterification reaction; an interestification reaction; a transesterification reaction; a hydroxylation reaction; and a condensation reaction following a hydroxylation reaction, whereby an oil-based product is produced.

[0017] In some cases, the oil-based product is selected from soap or a fuel product. In some example~~embodiments~~, the oil-based product is a fuel product selected from the group consisting biodiesel, renewable diesel, and jet fuel. In some cases, the fuel product is biodiesel with one or more of the following attributes: (i) 0.01- 0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.01 mcg/g, less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.01 mcg/g, less than 0.005 mcg/g, preferably less than 0.003 mcg/g, beta carotene; (iv) 0.01-0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v) 1-500 mcg/g, 35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 1%, less than 0.5%, preferably less than 0.25%, brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 100-500 mcg/g, 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.001-0.1 mcg/g, 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 10-500 mcg/g, 50-300 mcg/g, preferably 60.8-261.7 mcg/g, tocopherols. In some cases, the fuel product is renewable diesel that has a T10-T90 of at least 20°C, 40°C or 60°C. In some cases, the fuel product is jet fuel that meets HRJ-5 and/or ASTM specification D1655.

[0018] In an eleventh example~~aspect~~, described is~~the present invention provides~~ a triglyceride oil comprising (a) a lipid profile of at least 3% C8:0, at least 4% C10:0, at least 13% C12:0, at least 10% C14:0, and/or at least 60% saturated fatty acids, and (b) one or more of the following attributes: (i) 0.01-0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.01 mcg/g, less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.01 mcg/g, less than 0.005 mcg/g, prefereably less than 0.003 mcg/g, beta carotene; (iv) 0.01-0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v) 1-300 mcg/g, 35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 1%, less than 0.5%, preferably less than 0.25%, brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 100-500 mcg/g, 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.001-0.1 mcg/g, 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 10-500 mcg/g, 50-300 mcg/g, preferably 60.8-261.7 mcg/g, tocopherols.

[0019] In a twelvth exampleaspect, described isthe present invention provides an isolated oil from microalgae that has a C8:C10 fatty acid ratio of at least 5:1. In a related exampleaspect, described isthe present invention provides an isolated oil from microalgae with at least 50% to 75%, preferably at least 60%, saturated fatty acids. In another related exampleaspect, described isthe present invention provides an isolated oil from microalgae that has a C16:14 fatty acid ratio of about 2:1. In still another related exampleaspect, described isthe present invention provides an isolated oil from microalgae that has a C12:C14 fatty acid ratio of at least 5:1. In some examplembodiments, the microalgae contains at least one exogenous gene. In some cases, the microalgae is of the genus *Prototheca*.

[0020] In a thirteenth exampleaspect, described isthe present invention provides a triglyceride oil comprising (a) a lipid profile of less than 5% or less than 2%, preferably less than 1%, <C12; between 1%-10%, preferably 2%-7%, C14:0; between 20%-35%, preferably 23%-30%, C16:0; between 5%-20%, preferably 7%-15%, C18:0; between 35-60%, preferably 40-55%, C18:1; and between 1%-20%, preferably 2-15%, C18:2 fatty acids; and (b) one or more of the following attributes: (i) 0.01-0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.05-0.244 mcg/g, total carotenoids; (ii) less than 0.01 mcg/g, less than 0.005 mcg/g, preferably less than 0.003 mcg/g, lycopene; (iii) less than 0.01 mcg/g, less than 0.005 mcg/g, preferably less than 0.003 mcg/g, beta carotene; (iv) 0.01-0.5 mcg/g, 0.025-0.3 mcg/g, preferably 0.045-0.268 mcg/g, chlorophyll A; (v) 1-300 mcg/g, 35-175 mcg/g, preferably 38.3-164 mcg/g, gamma tocopherol; (vi) less than 1%, less than 0.5%, preferably less than 0.25%, brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) 100-500 mcg/g, 225-350 mcg/g, preferably 249.6-325.3 mcg/g, total tocotrienols; (viii) 0.001-0.1 mcg/g, 0.0025-0.05 mcg/g, preferably 0.003-0.039 mcg/g, lutein; or (ix) 10-500 mcg/g, 50-300, preferably 60.8-261.7 mcg/g, tocopherols.

[0021] In some cases, the triglyceride oil is isolated from a microbe comprising one or more exogenous gene. In some examplembodiments, the one or more exogenous gene encodes a fatty acyl-ACP thioesterase. In some cases, the fatty acyl-ACP thioesterase has hydrolysis activity towards fatty acyl-ACP substrates of chain length C14. In some examplembodiments, the microbe further comprises a mutated endogenous desaturase gene, wherein the mutation renders the gene or desaturase inactive.

[0022] In a fourteenth exampleaspect, described isthe present invention provides a method of producing a triglyceride oil comprising a lipid profile of less than 5%, or less than 2%, preferably less than 1%, <C12; between 1%-10%, preferably 2%-7%, C14:0; between 20%-35%, preferably 23%-30%, C16:0; between 5%-20%, preferably 7%-15%, C18:0; between 35%-60%, preferably 40-55%, C18:1; and between 1%-20%, preferably 2-15%, C18:2 fatty acids,

wherein the triglyceride oil is isolated from a microbe comprising one or more exogenous gene. In some cases, the triglyceride oil comprises a lipid profile of 1%-10%, preferably 3-5%, C14:0; 20%-30%, preferably 25-27%, C16:0; 5%-20%, preferably 10-15%, C18:0; and 35%-50%, preferably 40-45%, C18:1. In some example~~embodiments~~s, the one or more exogenous gene encodes a fatty acyl-ACP thioesterase. In some cases, the fatty acyl-ACP thioesterase has hydrolysis activity towards fatty acyl-ACP substrates of chain length C14. In some cases, the microbe further comprises a mutated endogenous desaturase gene, wherein the mutation renders the gene or desaturase inactive. In some cases, the one or more exogenous gene is a sucrose invertase. In some example~~embodiments~~s, the mutated endogenous desaturase gene is a stearoyl-acyl carrier protein desaturase (SAD) (*e.g.*, SEQ ID NOs: 199-200). In some example~~embodiments~~s, the mutated endogenous desaturase gene is a fatty acid desaturase (FAD).

[0023] In a fifteenth example~~aspect~~, described is~~the present invention provides~~ a oleaginous microbial cell, preferably a microalgal cell, comprising a triglyceride oil, wherein the fatty acid profile of the triglyceride oil is selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids. In some cases, the oleaginous microbial cell comprises an exogenous gene, and optionally, an endogenous desaturase of the oleaginous microbial cell has been inactivated or mutated to have less enzymatic activity.

[0024] In some cases, the fatty acid profile of the triglyceride oil is similar to the fatty acid profile of a naturally occurring oil. In some cases, the naturally occurring oil is selected from the group consisting of cocoa butter, coconut oil, palm oil, palm kernel oil, shea butter, beef tallow and lard. In some cases, the fatty acid profile of the triglyceride oil comprises a profile selected from the group consisting of, the total combined amounts of C8:0 and C10:0 is at least about 10%, the total combined amount of C10:0, C12:0, and C14:0 is at least about 50%, the total combined amount of C16:0, C18:0 and C18:1 is at least about 60%, the total combined amount of C18:0, C18:1 and C18:2 is at least about 60%, the total combined amount of C14:0, C16:0, C18:0 and C18:1 is at least about 60%, and the total combined amount of C18:1 and C18:2 is less than about 30%. In some cases, the fatty acid profile of the triglyceride oil comprises a ratio of fatty acids selected from the group consisting of C8:0 to C10:0 ratio of at least about 5 to 1, C10:0 to C12:0 ratio of at least about 6 to 1, C12:0 to C14:0 ratio of at least about 5 to 1, C14:0 to C12:0 ratio of at least about 7:1, and C14:0 to C16:0 ratio of at least about 1 to 2.

[0025] In some cases, the endogenous desaturase is selected from the group consisting of stearoyl ACP desaturase and delta 12 fatty acid desaturase. In some cases, the exogenous gene is selected from the group consisting of a gene encoding an acyl-ACP thioesterase. In some cases, the exogenous gene encodes an acyl-ACP thioesterase selected from the group consisting of those identified in Table 4. In some cases, the oleaginous microbial cell further comprises a gene encoding a sucrose invertase.

[0026] In various example~~embodiment~~s, the oleaginous microbial cell is a cell of a microalgal genus or species selected from *Achnanthes orientalis, Agmenellum, Amphiprora hyaline, Amphora coffeiformis, Amphora coffeiformis linea, Amphora coffeiformis punctata, Amphora coffeiformis taylori, Amphora coffeiformis tenuis, Amphora delicatissima, Amphora delicatissima capitata, Amphora sp., Anabaena, Ankistrodesmus, Ankistrodesmus falcatus, Boekelovia hooglandii, Borodinella sp., Botryococcus braunii, Botryococcus sudeticus, Carteria, Chaetoceros gracilis, Chaetoceros muelleri, Chaetoceros muelleri subsalsum, Chaetoceros sp., Chlorella anitrata, Chlorella Antarctica, Chlorella aureoviridis, Chlorella candida, Chlorella capsulate, Chlorella desiccate, Chlorella ellipsoidea, Chlorella emersonii, Chlorella fusca, Chlorella fusca var. vacuolata, Chlorella glucotropha, Chlorella infusionum, Chlorella infusionum var. actophila, Chlorella infusionum var. auxenophila, Chlorella kessleri, Chlorella lobophora (strain SAG 37.88), Chlorella luteoviridis, Chlorella luteoviridis var. aureoviridis, Chlorella luteoviridis var. lutescens, Chlorella miniata, Chlorella minutissima, Chlorella mutabilis, Chlorella nocturna, Chlorella parva, Chlorella photophila, Chlorella pringsheimii, Chlorella protothecoides (including any of UTEX strains 1806, 411, 264, 256, 255, 250, 249, 31, 29, 25, and CCAP strains 211/17 and 211/8d), Chlorella protothecoides var. acidicola, Chlorella regularis, Chlorella regularis var. minima, Chlorella regularis var. umbricata, Chlorella reisiglii, Chlorella saccharophila, Chlorella saccharophila var. ellipsoidea, Chlorella salina, Chlorella simplex, Chlorella sorokiniana, Chlorella sp., Chlorella sphaerica, Chlorella stigmatophora, Chlorella vanniellii, Chlorella vulgaris, Chlorella vulgaris, Chlorella vulgaris f. tertia, Chlorella vulgaris var. autotrophica, Chlorella vulgaris var. viridis, Chlorella vulgaris var. vulgaris, Chlorella vulgaris var. vulgaris f. tertia, Chlorella vulgaris var. vulgaris f. viridis, Chlorella xanthella, Chlorella zofingiensis, Chlorella trebouxioides, Chlorella vulgaris, Chlorococcum infusionum, Chlorococcum sp., Chlorogonium, Chroomonas sp., Chrysosphaera sp., Cricosphaera sp., Cryptomonas sp., Cyclotella cryptica, Cyclotella meneghiniana, Cyclotella sp., Dunaliella sp., Dunaliella bardawil, Dunaliella bioculata, Dunaliella granulate, Dunaliella maritime, Dunaliella minuta, Dunaliella parva, Dunaliella peircei, Dunaliella*

primolecta, Dunaliella salina, Dunaliella terricola, Dunaliella tertiolecta, Dunaliella viridis, Dunaliella tertiolecta, Eremosphaera viridis, Eremosphaera sp., Ellipsoidon sp., Euglena, Franceia sp., Fragilaria crotonensis, Fragilaria sp., Gleocapsa sp., Gloeothamnion sp., Hymenomonas sp., Isochrysis aff. galbana, Isochrysis galbana, Lepocinclis, Micractinium, Micractinium (UTEX LB 2614), Monoraphidium minutum, Monoraphidium sp., Nannochloris sp., Nannochloropsis salina, Nannochloropsis sp., Navicula acceptata, Navicula biskanterae, Navicula pseudotenelloides, Navicula pelliculosa, Navicula saprophila, Navicula sp., Nephrochloris sp., Nephroselmis sp., Nitschia communis, Nitzschia alexandrina, Nitzschia communis, Nitzschia dissipata, Nitzschia frustulum, Nitzschia hantzschiana, Nitzschia inconspicua, Nitzschia intermedia, Nitzschia microcephala, Nitzschia pusilla, Nitzschia pusilla elliptica, Nitzschia pusilla monoensis, Nitzschia quadrangular, Nitzschia sp., Ochromonas sp., Oocystis parva, Oocystis pusilla, Oocystis sp., Oscillatoria limnetica, Oscillatoria sp., Oscillatoria subbrevis, Pascheria acidophila, Pavlova sp., Phagus, Phormidium, Platymonas sp., Pleurochrysis carterae, Pleurochrysis dentate, Pleurochrysis sp., Prototheca wickerhamii, Prototheca stagnora, Prototheca portoricensis, Prototheca moriformis, Prototheca zopfii, Pyramimonas sp., Pyrobotrys, Sarcinoid chrysophyte, Scenedesmus armatus, Spirogyra, Spirulina platensis, Stichococcus sp., Synechococcus sp., Tetraedron, Tetraselmis sp., Tetraselmis suecica, Thalassiosira weissflogii, and *Viridiella fridericiana.*

[0027] In some cases, the oleaginous microbial cell is a cell of the genus *Prototheca*. In some cases, the oleaginous microbial cell is a cell of the genus *Prototheca moriformis*.

[0028] In some cases, the oleaginous microbial cell is an oleaginous yeast cell. In some cases, the oleaginous microbial cell is an oleaginous bacterial cell.

[0029] In some cases, the naturally occurring oil is cocoa butter and the exogenous gene comprises a *Carthamus tinctorus* thioesterase gene. In some cases, the naturally occurring oil is coconut oil. In some cases, the naturally occurring oil is palm oil and the exogenous gene comprises a *Elaeis guiniensis* thioesterase gene, a *Cuphea hookeriana* thioesterase gene, a combination of a *Cuphea hookeriana* KAS IV gene and a *Cuphea wrightii* FATB2 gene, or a construct designed to disrupt an endogenous KAS II gene. In some cases, the naturally occurring oil is palm kernel oil and the exogenous gene comprises a combination of a *Cuphea wrightii* FATB2 gene and a construct designed to disrupt an endogenous SAD2B gene. In some cases, the naturally occurring oil is shea butter. In some cases, the naturally occurring oil is beef tallow. In some cases, the naturally occurring oil is lard and the exogenous gene comprises a combination of *U. californica* thioesterase gene and a construct designed to

disrupt an endogenous SAD2B gene, a combination of a *Garcinia mangostana* thioesterase gene and a construct designed to disrupt an endogenous SAD2B gene, a *Brassica napus* thioesterase gene, or a *Cuphea hookeriana* thioesterase gene..

[0030] In a sixteenth exampleaspect, described isthe present invention provides an oleaginous microbial triglyceride oil composition, wherein the fatty acid profile of the triglyceride oil is selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids. In various examplembodiments, the triglyceride oil composition is produced by cultivating a population of oleaginous microbial cells or recombinant oleaginous microbial cells in a culture medium, wherein the oleaginous microbial cells are as described above, in particular those described above in connection with the fifteeth exampleaspect of the invention.

[0031] In some cases, the oleaginous microbial triglyceride oil composition further comprises an attribute selected from the group consisting of: (i) less than 0.3 mcg/g total carotenoids; (ii) less than 0.005 mcg/g lycopene; (iii) less than 0.005 mcg/g beta carotene; (iv) less than 0.3 mcg/g chlorophyll A; (v) less than 175 mcg/g gamma tocopherol; (vi) less than 0.25% brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) less than 350 mcg/g total tocotrienols; (viii) less than 0.05 mcg/g lutein; or (ix) less than 275 mcg/g tocopherols.

[0032] In a seventeenth exampleaspect, described isthe present invention provides a method of producing an oleaginous microbial triglyceride oil composition having a fatty acid profile selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids, wherein the method comprises the steps of: (a) cultivating a population of oleaginous microbial cells in a culture medium until at least 10% of the dry cell weight of the oleaginous microbial cells is triglyceride oil; and (b) isolating the triglyceride oil composition from the oleaginous microbial cells. In various examplembodiments, the triglyceride oil composition is produced via cultivation of a population of oleaginous microbial cells or recombinant oleaginous microbial cells as described above, in particular those described above in connection with the fifteenth exampleaspect of the invention.

[0033] In an eighteenth exampleaspect, described isthe present invention provides a method of making an oil-based product, wherein the method comprises the steps of: (a) subjecting the oleaginous microbial triglyceride oil composition, as described above in connection with the sixteenth

exampleaspect of the invention, to at least one chemical reaction selected from the group consisting of: saponification; metathesis; acid hydrolysis; alkaline hydrolysis; enzymatic hydrolysis; catalytic hydrolysis; hot-compressed water hydrolysis; a catalytic hydrolysis reaction wherein the lipid is split into glycerol and fatty acids; an amination reaction to produce fatty nitrogen compounds; an ozonolysis reaction to produce mono- and dibasic-acids; a triglyceride splitting reaction selected from the group consisting of enzymatic splitting and pressure splitting; a condensation reaction that follows a hydrolysis reaction; a hydroprocessing reaction; a hydroprocessing reaction and a deoxygenation reaction or a condensation reaction prior to or simultaneous with the hydroprocessing reaction; a gas removal reaction; a deoxygenation reaction selected from the group consisting of a hydrogenolysis reaction, hydrogenation, a consecutive hydrogenation-hydrogenolysis reaction, a consecutive hydrogenolysis-hydrogenation reaction, and a combined hydrogenation-hydrogenolysis reaction; a condensation reaction following a deoxygenation reaction; an esterification reaction; an interestification reaction; a transesterification reaction; a hydroxylation reaction; and a condensation reaction following a hydroxylation reaction; and (b) isolating the product of the reaction from the other components.

[0034] In some cases, the oil-based product is selected from the group consisting of a soap, a fuel, a dielectric fluid, a hydraulic fluid, a plasticizer, a lubricant, a heat transfer fluid, and a metal working fluid. In some cases, the oil-based product is a fuel product selected from the group consisting of: (a) biodiesel; (b) renewable diesel; and (c) jet fuel.

[0035] In some cases, the fuel product is biodiesel with one or more of the following attributes: (i) less than 0.3 mcg/g total carotenoids; (ii) less than 0.005 mcg/g lycopene; (iii) less than 0.005 mcg/g beta carotene; (iv) less than 0.3 mcg/g chlorophyll A; (v) less than 175 mcg/g gamma tocopherol; (vi) less than 0.25% brassicasterol, campesterol, stignasterol, or beta-sitosterol; (vii) less than 350 mcg/g total tocotrienols; (viii) less than 0.05 mcg/g lutein; or (ix) less than 275 mcg/g tocopherols.

[0036] In some cases, the fuel product is renewable diesel that has a T10-T90 of at least 20°C, 40°C or 60°C.

[0037] In some cases, the fuel product is jet fuel that meets HRJ-5 and/or ASTM specification D1655.

[0038] In another exampleaspect, the oleaginous microbial cell described hereinof the invention is edible. The triglyceride oils of the invention are also edible. In some cases, the microbial strain is cultivated and processed under good manufacturing process (GMP) conditions. As a food or a food ingredient, the oleaginous microbial cell can be consumed whole. Alternatively, the

microbial biomass is processed into a microbial flakes, powder or flour. Microbial flour is prepared by completely or partially lysing the cells in the form of a powder. When processed into microbial flour, the average particle size of lysed microbial biomass is between about 1 to 30 μm. The lysed microbial cells can agglomerate to form bigger particles of up to 1,000 μm. In one example~~embodiment~~, the flour further comprises a flow agent, antioxidants and the like. The microbial cells and the microbial oils described herein~~of the present invention~~ can be consumed by itself. Alternatively, the microbial cells and the microbial oils of the present invention can be combined with at least one other ingredient. By way of example, the microbial cells and the microbial oils can be combined with edible ingredients, e.g., egg, egg products, milk, dairy products, meats, grains, other edible fats, natural sweetners, artificial sweetners, etc. The microbial cells and the microbial oils can also be combined with preservatives and other ingredients added to processed foods.

[0039] All of the processes described herein can be performed in accordance with GMP or equivalent regulations. In the United States, GMP regulations for manufacturing, packing, or holding human food are codified at 21 C.F.R. 110. These provisions, as well as ancillary provisions referenced therein, are hereby incorporated by reference in their entirety for all purposes. GMP conditions in the Unites States, and equivalent conditions in other jurisdictions, apply in determining whether a food is adulterated (the food has been manufactured under such conditions that it is unfit for food) or has been prepared, packed, or held under unsanitary conditions such that it may have become contaminated or otherwise may have been rendered injurious to health. GMP conditions can include adhering to regulations governing: disease control; cleanliness and training of personnel; maintenance and sanitary operation of buildings and facilities; provision of adequate sanitary facilities and accommodations; design, construction, maintenance, and cleanliness of equipment and utensils; provision of appropriate quality control procedures to ensure all reasonable precautions are taken in receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing food products according to adequate sanitation principles to prevent contamination from any source; and storage and transportation of finished food under conditions that will protect food against physical, chemical, or undesirable microbial contamination, as well as against deterioration of the food and the container.

[0040] In some example~~embodiments~~, the oleaginous microbial cells are algal cells of a species of the genus *Chlorella* or *Prototheca*. In one example~~embodiment~~, the algae is *Chlorella protothecoides*. In another example~~embodiment~~, the algae is *Prototheca moriformis*. In some cases,

the biomass is derived from an algae that is a color mutant with reduced color pigmentation compared to the strain from which it was derived. Mutants with reduced color pigmentation are typically prepared using standard mutagenesis techniques. There are many fee-for-service laboratories that will generate mutants with reduced color pigmentation. In some examplembodiments, the microbial biomass is prepared by cultivating the microorganism heterotrophically and optionally in the absence of light.

[0041] In one examplembodiment, the microalgal strain is *Chlorella protothecoides* 33-55, deposited on October 13, 2009 at the American Type Culture Collection under deposit designation PTA-10397. In one examplembodiment, the microalgal strain is *Chlorella protothecoides* 25-32, deposited on October 13, 2009 at the American Type Culture Collection under deposit designation PTA-10396. In some cases, the microalgal strain providing the biomass has been grown and processed under good manufacturing process (GMP) conditions.

[0042] In another exampleaspect, described arethe present invention provides methods for preparing recombinant oleaginous microbial biomass suitable for use as a foodstuff. In these methods, the recombinant oleaginous microbes are fermented under heterotrophic conditions and so lack or have a significantly reduced amount of green pigment that characterizes other recombinant microalgal derived foodstuffs. In one examplembodiment, the recombinant oleaginous microbes lack or have significantly reduced amounts of any pigment. One example describesaspect of the invention provides recombinant microalgae of the genus *Chlorella* and *Protothecoides*. In some examplembodiments, disclosedthe invention is a microalgae of the genus recombinant *Protothecoides*. Another examplemboiment provides recombinant *Protothecoides moriformis* for use in foods and food ingredients. In one examplembodiment, the fermentation conditions are manipulated to provide a biomass rich in lipid. In another examplembodiment, the fermentation conditions are manipulated to provide a biomass rich in protein. In all examplembodiments, the methods can be carried out cheaply and efficiently at large scale (biomass produced in 4500 L or larger fermentors).

[0043] In another exampleaspect, described isthe present invention provides recombinant oleaginous microbial biomass, preferably microalgal biomass, suitable for incorporation into human foodstuffs. In one examplembodiment, this recombinant microbial biomass is the concentrated biomass resulting directly from the biomass preparation methods described hereinof the present invention. In another eexamplembodiment, this biomass is in the form of dried flakes resulting from drying, *e.g.*, drum drying, such biomass preparations. In this latter examplembodiment, an antioxidant can be added to the biomass prior to the drying step to extend the shelf-life of the biomass and any food product containing such biomass.

[0044] Yet another example describesaspect of the invention provides methods for further processing the biomass into flakes or a homogenate. In one examplembodiment, the dried flakes are rehydrated in deionized water to create a suspension. This suspension is then micronized with a high pressure homogenizer so that the average particle size is less than 20 μm, preferably 10 μm in size, creating a homogenate.

[0045] A further example describesaspect of the present invention provides methods for processing the recombinant oleaginous microbial biomass, preferably microalgal biomass, into a food ingredient that is multifunctional in that it provides healthy oils to foods and provides structural benefits to foods such as baked goods. In one examplembodiment, the processing involves pneumatic drying (*e.g.*, spray drying or flash drying) the biomass preparation to form a powder that contains a large percentage of intact recombinant cells. In another examplembodiment, the biomass is first micronized to disrupt the cells before pneumatic drying to form a flour that contains only a small percentage (or no) intact cells; in some embodiments a flow or dispersal agent is added prior to the drying step.

[0046] In yet another exampleaspect, describedthe present invention is directed to a method of producing an oil or oil-containing recombinant oleaginous microbial biomass suitable for human consumption. In some examplembodiments, the process involves extracting the lipid (triglyceride) from the biomass to form an oil. In one examplembodiment, the method comprises providing a microorganism, and culturing the microorganism in the presence of a feedstock that is not derived from a food composition suitable for human consumption, in which the microorganism converts at least some portion of the feedstock into triglyceride oil. In some cases, the triglyceride oil comprises at least 50%, 60%, 70%, 80% or 90% C18:1 lipid.

[0047] Other examplesThe present invention further provides foods that incorporate a recombinant microbial powder, recombinant microbial flour, and/or recombinant microbial oil. In one examplembodiment, the food is a baked good, dressing, sauce, or mayonnaise in which, relative to the same food produced using conventional recipes, all or a portion of the egg or butter has been replaced by a recombinant microbial flour rich in oil. In another examplembodiment, the food is a powdered egg product containing a recombinant microbial flour rich in oil. In another examplembodiment, the food is a liquid egg product containing a microbial flour rich in oil. In another examplembodiment, the food is a liquid milk product containing microbial protein, fiber, and oil. In another examplembodiment, the food is a meat product in which, relative to previously available meat products, a portion or all (a meat substitute) of the meat has been replaced by a recombinant microbial flour, recombinant microbial powder, or recombinant microbial flake rich in protein.

[0048] Also described are~~The invention also provides~~ methods of inducing satiety by providing recombinant microbial foods or microbial food ingredients containing microbial fiber and optionally microbial protein and/or microbial oil.

[0049] These and other aspects ~~and~~, embodiments and examples~~of the invention~~ are described in the accompanying drawing, a brief description of which immediately follows, the detailed description of the invention below, and are exemplified in the examples below. Any or all of the features discussed above and throughout the application can be combined in various embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] Figure 1 shows a chromatogram of renewable diesel produced from *Prototheca* triglyceride oil.

DETAILED DESCRIPTION OF THE INVENTION

[0051] The present invention arises from the discovery that *Prototheca* and certain related microorganisms have unexpectedly advantageous properties for the production of oils, fuels, and other hydrocarbon or lipid compositions economically and in large quantities, as well as from the discovery of methods and reagents for genetically altering these microorganisms to improve these properties. The oils produced by these microorganisms can be used in the transportation fuel, oleochemical, and/or food and cosmetic industries, among other applications. Transesterification of lipids yields long-chain fatty acid esters useful as biodiesel. Other enzymatic and chemical processes can be tailored to yield fatty acids, aldehydes, alcohols, alkanes, and alkenes. In some applications, renewable diesel, jet fuel, or other hydrocarbon compounds are produced. The present invention also provides methods of cultivating microalgae for increased productivity and increased lipid yield, and/or for more cost-effective production of the compositions described herein.

[0052] This detailed description of the invention is divided into sections for the convenience of the reader. Section I provides definitions of terms used herein. Section II provides a description of culture conditions useful in the methods of the invention. Section III provides a description of genetic engineering methods and materials. Section IV provides a description of genetic engineering of microorganisms (*e.g.*,*Prototheca*) to enable sucrose utilization. Section V provides a description of genetic engineering of microorganisms (*e.g.*, *Prototheca*) to modify lipid biosynthesis. Section VI describes methods for making fuels and chemicals. Section VII describes methods for preparing recombinant microbial biomass. Section VIII describes methods for processing recombinant microbial biomass into finished food products. Section IX describes methods for combining recombinant microbial biomass

SEQ ID NO: 82
5' 6S region genomic donor sequence

GCTCTTCGCCGCCGCCACTCCTGCTCGAGCGCGCCCGCGCGTGCGCCGCCAGCGC
CTTGGCCTTTTCGCCGCGCTCGTGCGCGTCGCTGATGTCCATCACCAGGTCCATG
AGGTCTGCCTTGCGCCGGCTGAGCCACTGCTTCGTCCGGGCGGCCAAGAGGAGC
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GCCAGCATCATCTGGCTCTGCCGCACCGAGGCCGCCTCCAACTGGTCCTCCAGCA
GCCGCAGTCGCCGCCGACCCTGGCAGAGGAAGACAGGTGAGGGGGGTATGAATT
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CCGCACGCTTCTTTCCAGCACCGTGATGGCGCGAGCCAGCGCCGCACGCTGGCGC
TGCGCTTCGCCGATCTGAGGACAGTCGGGGAACTCTGATCAGTCTAAACCCCCTT
GCGCGTTAGTGTTGCCATCCTTTGCAGACCGGTGAGAGCCGACTTGTTGTGCGCC
ACCCCCCACACCACCTCCTCCCAGACCAATTCTGTCACCTTTTTGGCGAAGGCAT
CGGCCTCGGCCTGCAGAGAGGACAGCAGTGCCCAGCCGCTGGGGGTTGGCGGAT
GCACGCTCAGGTACC

SEQ ID NO: 83
Relevant expression construct for *Cinnamomum camphora* thioesterase
(βtub::neo::nitred::βtub::*C.camphora* TE::nitred)

CTTTCTTGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTT
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CTGCATGGGCGCTCCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCC
CCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGA
TCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGG
GCGCCTCTTCCTCTTCGTTTCAGTCACAACCCGCAAACTCTAGAATATCAATGATC
GAGCAGGACGGCCTCCACGCCGGCTCCCCCGCCGCCTGGGTGGAGCGCCTGTTC
GGCTACGACTGGGCCCAGCAGACCATCGGCTGCTCCGACGCCGCCGTGTTCCGCC
TGTCCGCCCAGGGCCGCCCCGTGCTGTTCGTGAAGACCGACCTGTCCGGCGCCCT
GAACGAGCTGCAGGACGAGGCCGCCCGCCTGTCCTGGCTGGCCACCACCGGCGT
GCCCTGCGCCGCCGTGCTGGACGTGGTGACCGAGGCCGGCCGCGACTGGCTGCT
GCTGGGCGAGGTGCCCGGCCAGGACCTGCTGTCCTCCCACCTGGCCCCCGCCGAG
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GCAATGCTGATGCACGGGAAGTAGTGGGATGGGAACACAAATGGAGGATCCCGC
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AGCGCGGCATACACCACAATAACCACCTGACGAATGCGCTTGGTTCTTCGTCCAT
TAGCGAAGCGTCCGGTTCACACACGTGCCACGTTGGCGAGGTGGCAGGTGACAA
TGATCGGTGGAGCTGATGGTCGAAACGTTCACAGCCTAGGGATATCGAATTCCTT
TCTTGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCC
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CTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCG
CCTCTTCCTCTTCGTTTCAGTCACAACCCGCAAACACTAGTATGGCCACCGCATCC
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CCATGCTGTTCGCCGTGATCACCACCATCTTCTCCGCCGCCGAGAAGCAGTGGAC
CAACCTGGAGTGGAAGCCCAAGCCCAACCCCCCCCAGCTGCTGGACGACCACTT
CGGCCCCCACGGCCTGGTGTTCCGCCGCACCTTCGCCATCCGCAGCTACGAGGTG
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GCCCTGAACCACGCCAAGTCCGTGGGCATCCTGGGCGACGGCTTCGGCACCACC
CTGGAGATGTCCAAGCGCGACCTGATCTGGGTGGTGAAGCGCACCCACGTGGCC
GTGGAGCGCTACCCCGCCTGGGGCGACACCGTGGAGGTGGAGTGCTGGGTGGGC
GCCTCCGGCAACAACGGCCGCCGCCACGACTTCCTGGTGCGCGACTGCAAGACC
GGCGAGATCCTGACCCGCTGCACCTCCCTGAGCGTGATGATGAACACCCGCACCC
GCCGCCTGAGCAAGATCCCCGAGGAGGTGCGCGGCGAGATCGGCCCCGCCTTCA
TCGACAACGTGGCCGTGAAGGACGAGGAGATCAAGAAGCCCCAGAAGCTGAAC
GACTCCACCGCCGACTACATCCAGGGCGGCCTGACCCCCCGCTGGAACGACCTG
GACATCAACCAGCACGTGAACAACATCAAGTACGTGGACTGGATCCTGGAGACC
GTGCCCGACAGCATCTTCGAGAGCCACCACATCTCCTCCTTCACCATCGAGTACC
GCCGCGAGTGCACCATGGACAGCGTGCTGCAGTCCCTGACCACCGTGAGCGGCG
GCTCCTCCGAGGCCGGCCTGGTGTGCGAGCACCTGCTGCAGCTGGAGGGCGGCA
GCGAGGTGCTGCGCGCCAAGACCGAGTGGCGCCCCAAGCTGACCGACTCCTTCC
GCGGCATCAGCGTGATCCCCGCCGAGTCCAGCGTGATGGACTACAAGGACCACG
ACGGCGACTACAAGGACCACGACATCGACTACAAGGACGACGACGACAAGTGA
CTCGAGGCAGCAGCAGCTCGGATAGTATCGACACACTCTGGACGCTGGTCGTGT
GATGGACTGTTGCCGCCACACTTGCTGCCTTGACCTGTGAATATCCCTGCCGCTTT
TATCAAACAGCCTCAGTGTGTTTGATCTTGTGTGTACGCGCTTTTGCGAGTTGCTA
GCTGCTTGTGCTATTTGCGAATACCACCCCCAGCATCCCCTTCCCTCGTTTCATAT
CGCTTGCATCCCAACCGCAACTTATCTACGCTGTCCTGCTATCCCTCAGCGCTGCT
CCTGCTCCTGCTCACTGCCCCTCGCACAGCCTTGGTTTGGGCTCCGCCTGTATTCT
CCTGGTACTGCAACCTGTAAACCAGCACTGCAATGCTGATGCACGGGAAGTAGT
GGGATGGGAACACAAATGGAAAGCTT

SEQ ID NO: 84
3'6S region genomic donor sequence

GAGCTCCTTGTTTTCCAGAAGGAGTTGCTCCTTGAGCCTTTCATTCTCAGCCTCGA
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GTTGGTTCGTGCGTCTGGAACAAGCCCAGACTTGTTGCTCACTGGGAAAAGGACC
ATCAGCTCCAAAAAACTTGCCGCTCAAACCGCGTACCTCTGCTTTCGCGCAATCT
GCCCTGTTGAAATCGCCACCACATTCATATTGTGACGCTTGAGCAGTCTGTAATT
GCCTCAGAATGTGGAATCATCTGCCCCCTGTGCGAGCCCATGCCAGGCATGTCGC

WHAT IS CLAIMED IS:

1. A food composition comprising at least 0.1% w/w recombinant algal biomass and one or more other ingredient, wherein the recombinant algal biomass comprises at least about 10% triglyceride oil by weight and is cultured under heterotrophic conditions.

2. The food composition of claim 1, wherein the triglyceride oil comprises a fatty acid profile selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids.

3. The food composition of claim 1, wherein the fatty acid profile of algal triglyceride oil is similar to the fatty acid profile of a naturally occurring oil.

4. The food composition of claim 3, wherein the naturally occurring oil is selected from the group consisting of cocoa butter, coconut oil, palm oil, beef tallow, and lard.

5. The food composition of claim 1, wherein the fatty acid profile of the algal triglyceride oil is at least 60% C18:1.

6. The food composition of claim 5, wherein the fatty acid profile of the algal triglyceride oil is at least 80% C18:1.

7. The food composition of claim 1, wherein the recombinant algal biomass is predominantly intact cells.

8. The food composition of claim 1, wherein the recombinant algal biomass is predominantly lysed cells.

9. The food composition of claim 8, wherein the recombinant algal biomass is a homogenate.

10. The food composition of claim 8, wherein the recombinant algal biomass is a powder.

11. The food composition of claim 8, wherein the recombinant algal biomass is in the form of a powder, and wherein the recombinant algal biomass comprises at least about 40% triglyceride oil by dry weight.

12. The food composition of claim 1, that is a salad dressing, egg product, baked good, bread, bar, snack chip, pasta, sauce, soup, beverage, frozen dessert, butter or spread.

13. The food composition of claim 10, wherein average particle size of the powder is from about 0.2 to about 20 microns.

14. The food composition of claim 1, wherein the recombinant algal biomass is a species of the genus *Chlorella* or *Prototheca.*

15. The food composition of claim 14, wherein the recombinant algal biomass is selected from the group consisting of *Chlorella protothecoides* and *Prototheca moriformis.*

16. A method of making a food composition comprising combining heterotrophically cultivated recombinant algal biomass comprising at least 10% triglyceride oil by weight with at least one other ingredient.

17. The method of claim 16, wherein said triglyceride oil comprises a fatty acid profile selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids.

18. The method of claim 16 comprising the steps of:

a. determining the amount non-algal oil, non-algal fat or egg in a conventional food product; and

b. replacing a portion of the non-algal oil, non-algal fat or egg or supplementing the non-algal oil, non-algal fat or egg with a specified amount of recombinant algal biomass.

19. The method of claim 18, wherein non-algal oil, non-algal fat or egg is not added to the food composition.

20. The method of claim 18, wherein the amount of recombinant algal biomass is from about 0.25 times to about 4 times the mass or volume of the non-algal oil, non-algal fat or egg in the conventional food product.

21. The method of claim 18, wherein the recombinant algal biomass is predominantly lysed and is in the form of a powder or a homogenate.

22. The method of claim 16, wherein the recombinant algal biomass is a species of the genus *Chlorella* or *Prototheca.*

23. The method of claim 22, wherein the recombinant algal biomass is selected from the group consisting of *Chlorella protothecoides* and *Prototheca moriformis.*

24. A food composition comprising at least about 0.1% w/w recombinant algal biomass and one or more other ingredient, wherein the recombinant algal biomass is a color mutant and comprises at least about 10% triglyceride oil by dry weight.

25. The food composition of claim 24, wherein the triglyceride oil comprises a fatty acid profile selected from the group consisting of at least about 1% C8:0, at least about 1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids..

26. The food composition of claim 24, wherein the recombinant algal biomass is a species of the genus *Chlorella* or *Prototheca.*

27. The food composition of claim 26, wherein the recombinant algal biomass is selected from the group consisting of *Chlorella protothecoides* and *Prototheca moriformis.*

28. The food composition of claim 24, wherein the recombinant algal biomass is cultured under GMP conditions.

29. A food composition comprising at least 0.1% w/w algal triglyceride oil and one or more other ingredient, wherein the algal triglyceride oil is isolated from recombinant algal biomass that is cultured under heterotrophic conditions.

30. The food composition of claim 29, wherein said triglyceride oil comprises a fatty acid profile selected from the group consisting of at least about 1% C8:0, at least about

1% C10:0, at least about 1% C12:0, at least about 2% C14:0, at least about 30% C16:0, at least about 5% C18:0, at least about 60% C18:1, less than about 7% C18:2, and at least about 35% saturated fatty acids.

31. The food composition of claim 29, wherein the profile of the recombinant algal triglyceride oil is similar to the triglyceride profile of a naturally occurring oil.

32. The food composition of claim 31, wherein the naturally occurring oil is selected from the group consisting of cocoa butter, coconut oil, palm oil, beef tallow, and lard.

33. The food composition of claim 29, wherein the profile of the algal triglyceride oil is at least 60% C18:1.

34. The food composition of claim 33, wherein the profile of the algal triglyceride oil is at least 80% C18:1.

35. The food composition of claim 29, wherein the recombinant algal biomass is a species of the genus *Chlorella* or *Prototheca.*

36. The food composition of claim 35, wherein the recombinant algal biomass is selected from the group consisting of *Chlorella protothecoides* and *Prototheca moriformis.*

37. The food composition of claim 29 that is a salad dressing, egg product, baked good, bread, bar, snack chip, pasta, sauce, soup, beverage, frozen dessert, butter or spread.

WHAT IS CLAIMED IS:

1. A microbial oil obtained from a recombinant microalga, wherein the fatty acid profile of the oil comprises at least 40% saturated fatty acid having C10, C12 and C14, and the oil further comprises sterols of the recombinant microalga, wherein the recombinant microalga has been genetically engineered to express at least one lipid pathway enzyme at an altered level compared to a non-engineered microalga, the lipid pathway enzyme being selected from the group consisting of a desaturase, a ketoacyl synthase, and an acyl-ACP thioesterase.

2. The microbial oil of claim 1, wherein the at least one lipid pathway enzyme is an endogenous lipid pathway enzyme that is expressed at a lower level compared to the non-engineered microalga.

3. The microbial oil of claim 2, wherein the at least one endogenous lipid pathway enzyme is a desaturase.

4. The microbial oil of claim 3, wherein the at least one endogenous lipid pathway enzyme is fatty acyl desaturase enzyme.

5. The microbial oil of claim 3, wherein the at least one endogenous lipid pathway enzyme is a stearoyl ACP desaturase-enzyme.

6. The microbial oil of claim 2, wherein the at least one endogenous lipid pathway enzyme is an acyl-ACP thioesterase.

7. The microbial oil of claim 2, wherein the at least one endogenous lipid pathway enzyme is a ketoacyl synthase.

8. The microbial oil of claim 7, wherein the at least one endogenous lipid pathway enzyme is a ketoacyl synthase II enzyme.

9. The microbial oil of claim 7, wherein the at least one endogenous lipid pathway enzyme is a ketoacyl synthase I enzyme.

10. The microbial oil of claim 7, wherein the at least one endogenous lipid pathway enzyme is a ketoacyl synthase IV enzyme.

11. The microbial oil of claim 1, wherein the at least one lipid pathway enzyme is expressed at a higher level compared to the non-engineered microalga.

12. The microbial oil of claim11, wherein the at least one lipid pathway enzyme is a ketoacyl synthase.

13. The microbial oil of claim 1, wherein the fatty acid profile of the oil has not been altered by further processing the microbial oil by one or more of esterification, distillation, fractionation, crystallization, and precipitation.

14. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 70% saturated fatty acid.

15. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 4% C10.

16. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 10% C12.

17. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 10% C14.

18. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 40% C14

19. The microbial oil of claim 1, wherein the fatty acid profile of the comprises at least 50% total combined amounts of C10:0, C12:0 and C14:0.

20. The microbial oil of claim 1, wherein the microbial oil is *Prototheca* oil or *Chlorella* oil.

21. The microbial oil of claim 20, wherein the microbial oil is *Prototheca moriformis* oil or *Chlorella protothecoides* oil.

22. A blended composition comprising a microbial oil of claim 1 and an oil from soy, rapeseed, canola, palm, palm kernel, coconut, corn, waste vegetable, Chinese tallow, olive,

sunflower, cottonseed, chicken fat, beef tallow, porcine tallow, microalgae, macroalgae, microbes, Cuphea, flax, peanut, choice white grease, lard, Camelina sativa, mustard seed, cashew nut, oats, lupine, kenaf, calendula, help, coffee, linseed (flax), hazelnut, euphorbia, pumpkin seed, coriander, camellia, sesame, safflower, rice, tung tree, cocoa, copra, opium poppy, castor beans, pecan, jojoba, macadamia, Brazil nuts, avocado, petroleum, or a distillate fraction of any of the preceding oils.

23. The oil of claim 1, wherein the oil has a C12:C14 fatty acid ratio of at least 5:1.

24. The oil of claim 1, wherein the oil has a C12-C14 level of over 49%.

25. The microbial oil of claim 1, wherein the fatty acid profile of the oil comprises at least 70% total combined amounts of C10:0, C12:0 and C14:0.