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(54) Title: PREPARATION OF OXIDATIVELY STABLE OIL WITH LONG CHAIN OMEGA-3 FATTY ACIDS

(57) Abstract: Extraction and separation of an oxidatively stable oil containing at least eicosapentaenoic acid ("EPA") and docosa-hexaenoic acid ("DHA"), and optionally docosapentaenoic acid ("DPA"), from a mix of a first seed that does not contain EPA or DHA, or optionally DPA, and a second seed that does contain EPA and DHA, and optionally DPA.



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PREPARATION OF OXIDATIVELY STABLE OIL WITH LONG CHAIN OMEGA-3 FATTY ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/199,393, filed July 31, 2015, entitled "PREPARATION OF OXIDATIVELY STABLE OIL WITH LONG CHAIN OMEGA-3 FATTY ACIDS" and U.S. Provisional Patent Application Serial No. 62/325,093, filed April 20, 2016, entitled "PREPARATION OF OXIDATIVELY STABLE OIL WITH LONG CHAIN OMEGA-3 FATTY ACIDS", which applications are hereby incorporated by reference herein in their entirety.

BACKGROUND

[0002] Inclusion of omega-3 long-chain polyunsaturated fatty acids such as eicosapentaenoic acid ("EPA", 20 carbon atoms:5 unsaturated sites), docosahexaenoic acid ("DHA", 22 carbon atoms:6 unsaturated sites), as well as docosapentaenoic acid ("DPA", 22 carbon atoms:5 unsaturated sites) in the human diet has been linked with numerous health-related benefits. These benefits may include prevention or reduction of coronary heart disease, hypertension, type-2 diabetes, renal disease, rheumatoid arthritis, ulcerative colitis and chronic obstructive pulmonary disease, and aiding brain development and growth.

[0003] A number of studies have also indicated that omega-3 polyunsaturated fatty acids may be beneficial in infant nutrition and development and in treating or preventing various mental disorders such as schizophrenia, attention deficit hyperactive disorder and Alzheimer's disease.

SUMMARY

[0004] The present disclosure relates to the extraction and separation of an oxidatively stable oil containing at least eicosapentaenoic acid ("EPA") and docosahexaenoic acid ("DHA"), and optionally docosapentaenoic acid ("DPA"), from a mix of a first seed that does not contain EPA or DHA, or optionally DPA, and a second seed that does contain EPA and DHA, and optionally DPA. The oil of the present disclosure may be used, for example, in human food applications, animal feed, pharmaceutical applications and cosmetic applications.

[0005] In one aspect of the present disclosure, disclosed is a method of preparing an oil comprising DHA and EPA, the method comprising the steps of a) obtaining a first seed and a second seed, wherein the first seed does not comprise DHA or EPA and wherein the second seed comprises DHA and EPA; b) mixing the first seed and the second seed in a ratio of about 1:9 to about 9:1 to provide a seed mix; and c) pressing the seed mix to provide the oil comprising DHA and EPA.

DESCRIPTION OF THE DRAWINGS

[0006] Figures 1 and 2 graphically demonstrate the oxidative stability of pressed oils of the present disclosure compared to the oxidative stability of oil blends.

[0007] Figures 3 and 4 graphically demonstrate the oxidative stability of pressed oils of the present disclosure containing antioxidants compared to the oxidative stability of oil blends containing antioxidants.

[0008] Figures 5 and 6 graphically demonstrate the oxidative stability of extracted meal oils of the present disclosure.

[0009] Figure 7 graphically demonstrates the color of pressed oils of the present disclosure.

[0010] Figure 8 graphically demonstrates the chlorophyll value of pressed oils of the present disclosure.

[0011] Figure 9 lists the fatty acid percentages in pressed oils of the present disclosure.

[0012] Figure 10 graphically demonstrates the oxidative stability index (“OSI”) of oil from canola seeds including DHA, EPA, and/or DPA (“tCanola”) and seeds not including DHA, EPA, and/or DPA (“Canola”), where the seeds were subjected to a 40 °C storage temperature for up to 120 days.

[0013] Figure 11 graphically demonstrates weight percentage of total saturated fats measured in the oils oil from canola seeds including DHA, EPA, and/or DPA (“tCanola”) and seeds not including DHA, EPA, and/or DPA (“Canola”), where the seeds were subjected to a 40 °C storage temperature for up to 120 days.

[0014] Figure 12 graphically demonstrates the weight percentage of DHA + EPA present in oil from seeds including DHA, EPA, and/or DPA (“tCanola”), where the seeds were subjected to a 40 °C storage temperature for up to 120 days.

DETAILED DESCRIPTION

[0015] In this application, the phrase “oil comprising DHA and EPA” means pressed oil, meal oil, or Refined Bleached Deodorized (“RBD”) oil containing eicosapentaenoic acid (“EPA”) and docosahexaenoic acid (“DHA”).

Method of Preparing Oils

[0016] As used herein, the term “seed” refers to the seed, bean, or nut of a plant from which oil can be expressed and/or extracted such as, for example, a rapeseed (*e.g.*, canola), a sunflower seed, a poppy seed, a sesame seed, a soy bean, a pistachio, a peanut, a cashew, a walnut, a pine nut, or an almond. Seeds useful in embodiments of the present application are available commercially, for example, from Cargill, Incorporated, Wayzata Minnesota, USA.

[0017] In some embodiments, the first seed (*i.e.*, the seed that does not comprise DHA and EPA) can include, for example, a rapeseed (*e.g.*, a canola seed). Canola seeds useful in embodiments of the present application are available commercially, for example, from Cargill, Incorporated, Wayzata Minnesota, USA. The second seed (*i.e.*, the seed that does comprise DHA and EPA) may be obtained from recombinant or transgenic plants constructed or made using reported transformation processes. Suitable recombinant or transgenic plants are reported, for example, in US 7,807,849, US Pub. No. 2005/0129739, EP 1 117 303B1 and EP 1 401 259B1, all incorporated by reference herein.

[0018] According to the present disclosure, modified or transformed canola seeds, for example, with increased levels of DHA and EPA, and optionally DPA, mixed with canola seeds with no or no significant levels of DHA, EPA, and/or DPA may be hot pressed/expelled to produce oxidatively stable crude oil without paint-like or fish-like aroma or odor. A paint-like or fish-like aroma or odor is generally considered undesirable as it may indicate an edible oil of low nutritional value and poor oxidative status, *i.e.*, an edible oil that is not fresh.

[0019] Oxidative stability of oils made from a mixture of seeds, some seeds including DHA, EPA, and/or DPA and other seeds not including DHA, EPA, and/or DPA, is surprisingly similar to oil not containing DHA, EPA and/or DPA, as one skilled in the art would expect an oil containing any of the oxidatively less stable polyunsaturated fatty acids such as DHA, EPA and DPA to be less stable when compared to an oil not containing such polyunsaturated fatty acids.

[0020] One embodiment of the present disclosure is a method of preparing an oil comprising DHA and EPA, the method comprising the steps of a) obtaining a first canola seed and a second canola seed, wherein the first canola seed does not comprise DHA or EPA and wherein the second canola seed comprises DHA and EPA (the second canola seed may optionally further comprises DPA); b) mixing the first canola seed and the second canola seed in a ratio of about 1:9 to about 9:1 to provide mixed seed; and c) pressing the mixed seed to provide the oil. In other embodiments, the ratio of first and second canola seeds may be in ranges of 1:4, 3:7, 2:3, 1:1, 3:2, 7:3, or 4:1. The ratio of first and second canola seed may be selected in order to provide an oil comprising about 0.1% to about 5% DHA, about 0.1% to about 15% of EPA, and optionally about 0.1% to about 15% DPA.

[0021] The second canola seed comprising DHA and EPA may be stable under storage conditions at 40 °C, demonstrating less than a 30%, less than a 29%, less than a 28%, less than a 27%, less than a 26%, or less than a 25% decrease in oxidative stability index (“OSI”) at 110 °C after storage for at least 120 days. In some embodiments, the second canola seed may further demonstrate no significant decrease in the weight percentage of total saturated

fats and/or in the weight percentage of DHA + EPA in the seed oil after storage for at least 120 days.

[0022] The mixed seed of step b. may be tempered using conventional, known processing conditions to provide mixed seed with a moisture content of about 5% to about 15%. Some embodiments of the present disclosure may include about 0.1% to about 5% DHA, about 0.1% to about 15% of EPA, and optionally about 0.1% to about 15% DPA.

[0023] In other embodiments of the disclosure, the mixed seed may be ground or flaked before pressing in order to provide either a ground or flaked mixed seed. In some embodiments, the ground or flaked mix seed may be heat conditioned. Heat conditioning, in part, inactivates undesired enzymes in the seed that well detrimentally affect the physical properties or characteristics of the resulting oil. Suitable heat conditioning conditions may include heating the flaked mixed seed at a temperature in the range of about 80 °C to about 110 °C for a period of time of about 20 minutes to about 90 minutes, as well as other heating methods or processes conventionally used in the oil processing industry.

[0024] In embodiments of the present disclosure, either the first or the second seed, or both the first and the second seed, may be treated with an antioxidant. Treatment with an antioxidant can occur before the first and second seed are combined, while the first and second seed are being combined, or after the first and second seed are combined. In some embodiments, the first seed may be treated with a first antioxidant and the second seed may be treated with a second antioxidant that is different than the first antioxidant. In some embodiments, the expressed oil, before or after further processing, may be treated with an antioxidant. The antioxidant added to the expressed oil may be the same antioxidant used to treat the first and/or the second seed or it may be a different antioxidant.

[0025] Suitable antioxidants may include, for example, at least one of tert-butyl hydroquinone ("TBHQ"), butylated hydroxytoluene ("BHT"), butylated hydroxyanisole ("BHA"), and/or propyl gallate, as well as other conventional, known agents added to foods containing fats and oils to prevent oxidation. One example of a suitable antioxidant is a

TBHQ and propyl glycol blend sold under the trade name GRINDOX 512 by Danisco (DuPont Nutrition & Health, Copenhagen, Denmark). Another example of a suitable antioxidant is a 0.3% rosemary and tocopherols blend sold by Naturex, Inc., Avignon, France. Another example of a suitable antioxidant is a 0.3% rosemary and citric acid blend sold by Kalsec, Inc., Kalamazoo, Michigan, USA.

[0026] In addition, further antioxidants may include a suitable synthetic antioxidant typically used in food, feed, pharmaceuticals, or cosmetics. A synthetic antioxidant, for example, may be dissolved in a water or oil solution (e.g., propyl glycol), and may be then sprayed onto seed at a concentration of less than about 200 ppm.

[0027] In other embodiments, pressing the oil further comprises hot pressing the mixed seed to provide a meal and solvent extracting the oil from the meal. In still other embodiments, the method may include grinding or flaking the mixed seed of step b. to provide ground or flaked mixed seed as well as heat conditioning the ground or flaked mixed seed to provide heat conditioned ground or flaked mixed seed by, for example, heating the flaked mixed seed at a temperature in the range of about 80 °C to about 110 °C for a period of time of about 20 minutes to about 90 minutes.

[0028] The oil of the present disclosure may be further treated or processed using conventional, known refining, bleaching and deodorizing treatments, such as the refining, bleaching, and deodorizing treatments described by Daun *et al. Canola: Chemistry, Production, Processing, and Utilization*; AOCS Press: Urbana, 2011.

[0029] In yet other embodiments, the DHA and EPA oil has an desirable physical properties or characteristics such as an oxidative stability index (“OSI”) per the American Oil Chemist’s Society Official Method Cd 12b-92 (revised 2013) of about at least about 4 hours for refined, bleached, and deodorized (“RBD”) oil and at least about 1 hour for pressed oil and meal oil, a stability determined by an accelerated oxidative stability test at 60°C (Schall oven test) of hydroperoxide formation (Peroxide Value (“PV”) AOCS Cd 8b-90), about less than 10 milliequivalents peroxide/kg of oil after 3 days at 60°C, less than 20 PV after 6 days at

60°C, less than 30 PV after 9 days at 60°C, less than 70 PV after 12 days at 60°C; a color of about less than 1 Red for RBD using AOCS Method # Cc 13b-45 (as reapproved 2009) and about less than 20 Red for pressed and meal oils that is measured according to AOCS Method Td 1a-64.

[0030] The oil of the present disclosure may be used in a variety of applications such as, for example, dietetic, pharmaceutical, or cosmetic compositions. Suitable compositions using oil of the present disclosure are reported in US 7,807,849, US Pub No 2005/0129739, EP 1 117 303B1 and EP 1 401 259B1, all incorporated by reference herein.

Oil Physical Properties and Characteristics

Oxidative Stability Index

[0031] Oxidative Stability Index (“OSI”) per the American Oil Chemist’s Society (“AOCS”) Official Method Cd 12b-92 (revised 2013) is a food industry accepted, edible oil parameter that defines oil resistance to oxidation. Commonly, the higher OSI value an oil has, the more stable the oil is thought to be. In order to compare OSI values of different samples, OSI testing of each sample must occur at the same temperature; OSI values determined at 110 °C are most common in the edible oil industry. OSI is used for prediction of oil stability during storage (*i.e.*, oil shelf life) and how oils may behave during food processing (e.g., frying, baking) and product storage (*i.e.*, product shelf life). A typical OSI value at 110°C for sunflower oil is 3-5 hours, and for soybean oil is 4-7 hours. The correlation of OSI values at 110 °C to physical characteristics of an oil are summarized in Table 1.

Table 1. Correlation of OSI values at 110 °C to Physical Characteristics of Oils

OSI at 110 °C	Physical Characteristics of Oil	Oil Examples
<3	Unstable Oils are susceptible to rapid oxidation and are considered unstable; they require special storage conditions (e.g., refrigeration, specialty packaging) and have a shelf life of <6 months	Linseed and fish oils
4-10	Moderately stable Product shelf life of 6-9 months	Commodity soybean, sunflower, and canola oils
10-20	Highly stable. Oil shelf life >12 months	High-oleic canola, sunflower, and soybean oils
>20	Very highly stable. Oil shelf life >18 months	Specialty oils including antioxidants and palm oil

Peroxide Value

[0032] Determination of hydroperoxide formation, i.e., Peroxide Value (“PV”), may be made using an accelerated oxidative stability test at 60°C (Schall oven test) according to AOCS Cd 8b-90).

Color

[0033] The color of RBD oils of the present disclosure may be readily determined using AOCS Official Method Cc 13b-45 (reapproved 2009) titled “Wesson Method Using Color Glasses Calibrated in Accordance with the AOCS-Tinometer Color Scale”. In this method, the color of a liquid oil is determined by comparing the color of the oil with the color of one or more standardized color glasses using particular colorimeters as specified by the published method. The color for pressed and meal oils (the “Gardner red” value) of the present disclosure may be readily determined using AOCS Method Td 1a-64.

[0034] Color is important quality characteristic of an oil because it correlates with oil freshness and composition. For example, red pigments formed during thermal oil degradation (e.g., during frying), may affect the final product color of baked goods and fried

foods. Typical industry color (red) specification (“R”) is less than about 1R for an RBD oil and less than about 20 R for pressed and meal oils.

Chlorophyll Level

[0035] Chlorophyll level is an important quality parameter for seed, pressed oil, meal oil and bleached oil. Chlorophyll is a group of green pigments and is important not only because it may affect the final appearance of a product, but also because it indicates the maturity of seeds, it can affect oil refining parameters (e.g., the amount of bleaching earth needed), and the oxidative stability of oils (chlorophyll is a prooxidant). For seeds and pressed oil, a typical chlorophyll specification is <30 ppm. For bleached oil, a typical chlorophyll specification is <30 ppb.

Fatty Acid Profile

[0036] In the Examples set forth below, unless otherwise indicated, the fats are analyzed via a gas chromatograph determination of fatty acid profile per AOCS Official Method Ce 1i-07.

EXAMPLES

Example 1. Oil Blend Preparation

Experimental Protocols

Grinding/oil expelling:

1. Blend a first seed comprising DHA and EPA and optionally DPA with a second seed not comprising DHA and EPA and optionally DPA to a desired ratio (*e.g.*, first seed:second seed ratio of 1:9 to 9:1) to provide a seed mix.
2. Grind the seed mix in a CUSTOM GRIND Coffee Grinder (Hamilton Beach Brands, Inc., Glen Allen, VA, United States) to provide a ground seed mix where most of the seed coat has been broken and very few whole seeds are visible. Avoid over grinding into a fine powder. For example, grind approximately 100 g of the seed mix for about 45 seconds using the grinder described above.
3. Transfer the ground seed mix to a stainless steel beaker (1 kg) and heat the ground seed mix with stirring at 85 °C for approximately 30 minutes.
4. Turn on cylinder electric heat of a KOMET Single Screw Vegetable Oil Expeller CA59G (IBG Monforts Oekotec, Mönchengladbach, Germany) about 10 minutes

prior to the start of pressing the ground seed mix. Leave the heater on while the ground seed mix is being pressed. Collect oil from pressing and place it in a centrifuge tube. Retain the ground, pressed seed mix (“meal”) for further processing (described below).

5. Centrifuge the pressed oil to separate fine protein material from the oil in a CENTRIFIC 225a centrifuge (Thermo Fisher Scientific Inc., Waltham, MA USA) at speed setting #6 for 5 minutes. Pipet the clear oil from the centrifuge tube for testing.
6. Test clear oil immediately or transfer it to a vial that has been purged with an inert gas, (*e.g.*, nitrogen), flush with and inert gas (*e.g.*, nitrogen), seal immediately, and refrigerate sample at 4 °C.

Meal Oil Extraction:

1. Combine meal (60g) with hexane (150 mL) in a flask (250 mL) to provide a meal mixture.
2. Shake the meal mixture in the flask for about one hour at room temperature (*e.g.*, 23 °C) with a Lab-line Multi-Wrist Shaker (available from Colonial Scientific, Richmond, VA, USA) on slow for 1 hour.
3. Filter the shaken meal mixture through Whatman #4 filter paper (Cole-Parmer, Vernon Hills, IL, USA).
4. Filtrates from duplicate flasks are combined and the hexane is evaporated using a ROTAVAPOR R215 rotary evaporator (Buchi Corporation, New Castle, DE, USA) at 60 °C, 100 rpm to provide approximately 16 g of oil for every 120 g of meal.

Example 2. Oil and Oil Blend Physical Characteristics and Properties

[0037] Canola oil Samples A and B were prepared from CV80 high oleic canola seeds without DHA and EPA, commercially available from Cargill, Incorporated, as described in Example 1.

[0038] Canola oil Samples C and D were prepared from mixed canola seeds, *i.e.*, 40 wt% CV80 high oleic canola seeds without DHA and EPA commercially available from Cargill,

Incorporated, plus 60 wt% of a canola seed including about 8.5 wt% EPA+DHA (“tCanola”). The oils were tested for the physical characteristics and properties listed in Table 2, below. The tests were made on the crude oils as well as oils that were refined, bleached, and deodorized according to the further treatment steps described above. For Sample C, 350lb of seed blend was prepared (60% tCanola blended with 40% CV80). For Sample D, 21 kg pressed tCanola oil were blended with 5kg CV80 crude oil (81%/19% blend). Seeds moisture was measured and then adjusted with water to achieve about 12% moisture. Seeds were blended with tumbler blender and tempered for at least 8 hours. Tempered seeds were flaked with roller flaker to achieve about 0.22mm flake thickness. Flakes were transferred to a FRENCH Model 324 Vertical, Stacked Conditioner (French Oil Mill Machinery Co., Piqua, OH, USA) and treated at 100 °C for 30 min. Hot conditioned flakes were immediately expeller pressed. Pressed oil was filtered, acid degummed with 500 ppm phosphoric acid, neutralized with caustic, washed, dried, and clay bleached. After refining, the oil was transferred to an FT68 deodorization unit (Armfield Limited, Ringwood, England) for deodorization at 245 °C for 80 minutes at less than 8 mbar vacuum.

[0039] As shown in Table 2, Sample oils A-D meet RBD oil quality specification of good quality RBD oil (free fatty acids (“FFA”), color, and chlorophyll). All oils have a clean aroma without any off notes. Sample oil C has an OSI at 110 °C of 8 hours and Sample oil D has an OSI at 110 °C of 4 hours, OSI hours comparable to commodity oils. Sample D was further stabilized by blending RBD high oleic canola oil (CV80) and antioxidants to achieve OSI hours of a high-stability oils, as shown in Table 3.

Table 2. Comparative Data for Oil Samples

Sample	Free Fatty Acids	Color (R)	Chlorophyll	DHA+EPA (wt%)
Sample A crude CV80	0.443		35 ppm	—
Sample A refined-bleached CV80	0.19	4.2	—	—
Sample A refined-bleached-bleached CV80	0.177	0.8	31 ppb	—
Sample A refined-bleached-deodorized CV80	0.01	0.8	—	—
Sample B crude CV80	0.476	6.8	25 ppm	—

Sample B refined-bleached CV80	0.069	1.4	14 ppb	—
Sample B refined-bleached-deodorized CV80	0.039	0.3	12 ppb	—
Sample C crude CV80/tCanola* (60/40)	0.443		—	—
Sample C refined-bleached CV80/tCanola* (60/40)	0.0983	3.7	10 ppb	—
Sample C refined-bleached-deodorized CV80/tCanola* (60/40)	0.0293	0.2	8 ppb	0.83
Sample D crude tCanola8/CV80 (81/19)	0.477	20	13 ppm	3.73
Sample D refined-bleached	0.115	3.7	—	—
Sample D refined-bleached-deodorized	0.04	0.9	—	2.15

*tCanola = DHA + EPA canola seed

Table 3: Stability of DHA/EPA RBD + CV80 RBD Oil Blends

DHA+EPA(mg)/14g oil	25	50	75	100	125	150	175	250
DHA+EPA (%)	0.2	0.4	0.5	0.7	0.9	1.1	1.3	1.8
032715C-RBD Sample5 RBD oil (%) in CV80	8.5	17.0	25.5	34.0	42.5	51.0	59.5	85.0
OSI (hr) at 110 °C for blends	15.6	12	10.14	7.96	7.46	6.49	5.89	4.16
OSI (hr) at 110 °C for blends with 0.3% Rosemary&Citric Acid (Kalsec)	49.68	39.27	29.72	28.22	23.55	21.9	20.67	13.63
OSI (hr) at 110 °C for blends with 0.3% Rosemary&Tocopherols (Naturex)	29.2	23.6	19.8	16.7	13.6	11.1	8.2	

Example 3. Oil and Oil Blend Physical Characteristics and Properties

[0040] Blends of CV80 high oleic canola seeds (“CV80”) or commodity canola seeds (“Canola”) without DHA and EPA, commercially available from Cargill, Incorporated, and canola seed including about 8.5 wt% EPA+DHA (“tCanola”) were prepared to the desired ratios of CV80 or Canola:tCanola, *i.e.*, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 to provide mixed seed. The seed mixes were individually ground/expelled and the resulting meals were extracted as described in Example 1 and are hereinafter referred to as “oils made from seed blends” (“SB”). For comparison, “oil blends” (“OB”) were prepared by combining CV80 oil or Canola oil with tCanola oil in ratios of CV80 or Canola oil:tCanola oil of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1.

[0041] Figure 1 is a graph of the oxidative stability index (“OSI”) at 110 °C of pressed oils made from CV80/tCanola seed blends (“SB”) and oil blends (“OB”). Figure 2 is a graph of the oxidative stability index (“OSI”) at 110 °C of pressed oils made from Canola/tCanola seed blends (“SB”) and oil blends (“OB”). Referring to Figures 1 and 2, oils having a DHA+EPA content of less than 5% prepared with CV80 (Figure 1, as well those oils prepared with Canola (Figure 2), surprisingly demonstrated oxidative stability typical of commodity canola oils, *i.e.*, 4-10 hours, or even the oxidative stability typical of high stability oils. *i.e.*, greater than 10 hours.

[0042] Figure 3 is a graph of the oxidative stability index (“OSI”) at 110 °C of pressed oils with added antioxidant (3000 ppm) made from CV80/tCanola seed blends (“SB”) and oil blends (“OB”). Figure 4 is a graph of the oxidative stability index (“OSI”) at 110 °C of pressed oils with added antioxidant (3000 ppm) made from CV80/tCanola seed blends (“SB”) and oil blends (“OB”). Referring to Figures 3 and 4, oils having DHA+EPA content of less than 8.5% prepared with CV80 (Figure 3), as well with Canola (Figure 4), with rosemary and ascorbic acid antioxidants added, demonstrated a strong response to antioxidants addition, *i.e.*, Samples with DHA+EPA content of 6.7-8.5% had oxidative stability typical of commodity oils, and samples with DHA+EPA content of less than 6% had oxidative stability typical of high-stability oils.

[0043] As shown in Figures 5 and 6, the oxidative stability index (“OSI”) at 110 °C of hexane-extracted meal oils made from seed blends (SB) meal prepared from CV80/tCanola (Figure 5) as well with Canola/tCanola (Figure 6) for oils with DHA+EPA content less than 8.5% had higher oxidative stability than the corresponding pressed oils. Measurements of color (Figure 7) and chlorophyll (Figure 8) of oils made from seed blends(SB) demonstrate that seeds used were of good quality (i.e., the seeds were mature seeds). As shown in Figure 9, the fatty acid composition of the pressed oils made from seed blends (“SB”) are similar to the fatty acid profiles of oils made by physical blending of two pressed oils (“OB”).

Example 4. Properties of Oils from Seeds Stored at 40 °C

[0044] Canola seeds including about 8.5 wt% DHA + EPA (“tCanola”) and commodity canola seeds not including DHA or EPA (“Canola”) were stored 40 °C in a forced-air oven without humidity control. Oils were pressed from the seeds as described in Example 1 after storage for up to 120 days. The oxidative stability index (“OSI”) at 110 °C, weight percentage of total saturated fats, and weight percentage of DHA + EPA were determined as described above.

[0045] Figure 10 is a graph of the oxidative stability index (“OSI”) at 110 °C of pressed oils made from Canola seeds and tCanola seeds. Referring to Figure 10, canola seeds having a DHA + EPA content of about 8.5% surprisingly demonstrated only about a 24% decrease in OSI after 120 days compared to a 32% decrease in OSI for canola seeds not including DHA and EPA over the same time period.

[0046] The weight percentage of total saturated fats present in the oils of the canola seeds were measured, as shown in Figure 11. Referring to Figure 11, no significant change in the percentage of saturated fats was observed over 120 days in either Canola or tCanola.

[0047] The weight percentage of DHA + EPA present in tCanola seeds were measured, as shown in Figure 12. Referring to Figure 12, no significant change in the weight percentage of DHA + EPA was observed over 120 days in tCanola.

CLAIMS

What is claimed is:

1. A method of preparing an oil comprising DHA and EPA, the method comprising:
 - a. obtaining a first canola seed and a second canola seed, wherein the first canola seed does not comprise DHA or EPA and wherein the second canola seed comprises DHA and EPA;
 - b. mixing the first canola seed and the second canola seed in a ratio of about 1:9 to about 9:1 to provide mixed seed; and
 - c. pressing the mixed seed to provide the oil comprising DHA and EPA.
2. The method of claim 1, wherein pressing the oil further comprises hot pressing the mixed seed to provide a meal and solvent extracting the oil from the meal.
3. The method of claim 1, further comprising tempering the mixed seed of step b. to provide mixed seed with a moisture content of about 5% to about 15%.
4. The method of claim 1, further comprising grinding or flaking the mixed seed of step b. to provide ground or flaked mixed seed.
5. The method of claim 4, further comprising heat conditioning the ground or flaked mixed seed to provide heat conditioned ground or flaked mixed seed.
6. The method of claim 5, wherein the heat conditioning comprises heating the flaked mixed seed at a temperature in the range of about 80 °C to about 110 °C for a period of time of about 20 minutes to about 90 minutes.
7. The method of claim 1, wherein at least one of the first canola seed and the second canola seed of step a. is treated with an antioxidant.
8. The method of claim 7, wherein the antioxidant comprises at least one of TBHQ, BHT, BHA, or propyl gallate.
9. The method of claim 1, wherein an antioxidant is added to the oil of step c.
10. The method of claim 1, wherein the second canola seed further comprises DPA.

11. The method of claims 1-10, wherein the oil has an oxidative stability index of at least about 10 hours.
12. The method of claims 1-10, wherein the oil comprises about 0.1% to about 5% DHA, about 0.1% to about 15% of EPA, and optionally about 0.1% to about 15% DPA.
13. The method of claim 1, wherein the oil is further refined, bleached, or deodorized.
14. The method of claim 13, wherein the oil has a color of about < 1 red using AOCS method # Cc 13b-45.
15. The method of claims 1-10, wherein the oil has a chlorophyll level of about less than 30 ppb.
16. A stable oil blend comprising DHA, EPA and DPA prepared by the method of claims 1-10.
17. A dietetic, pharmaceutical, or cosmetic composition comprising the oil of claim 16.

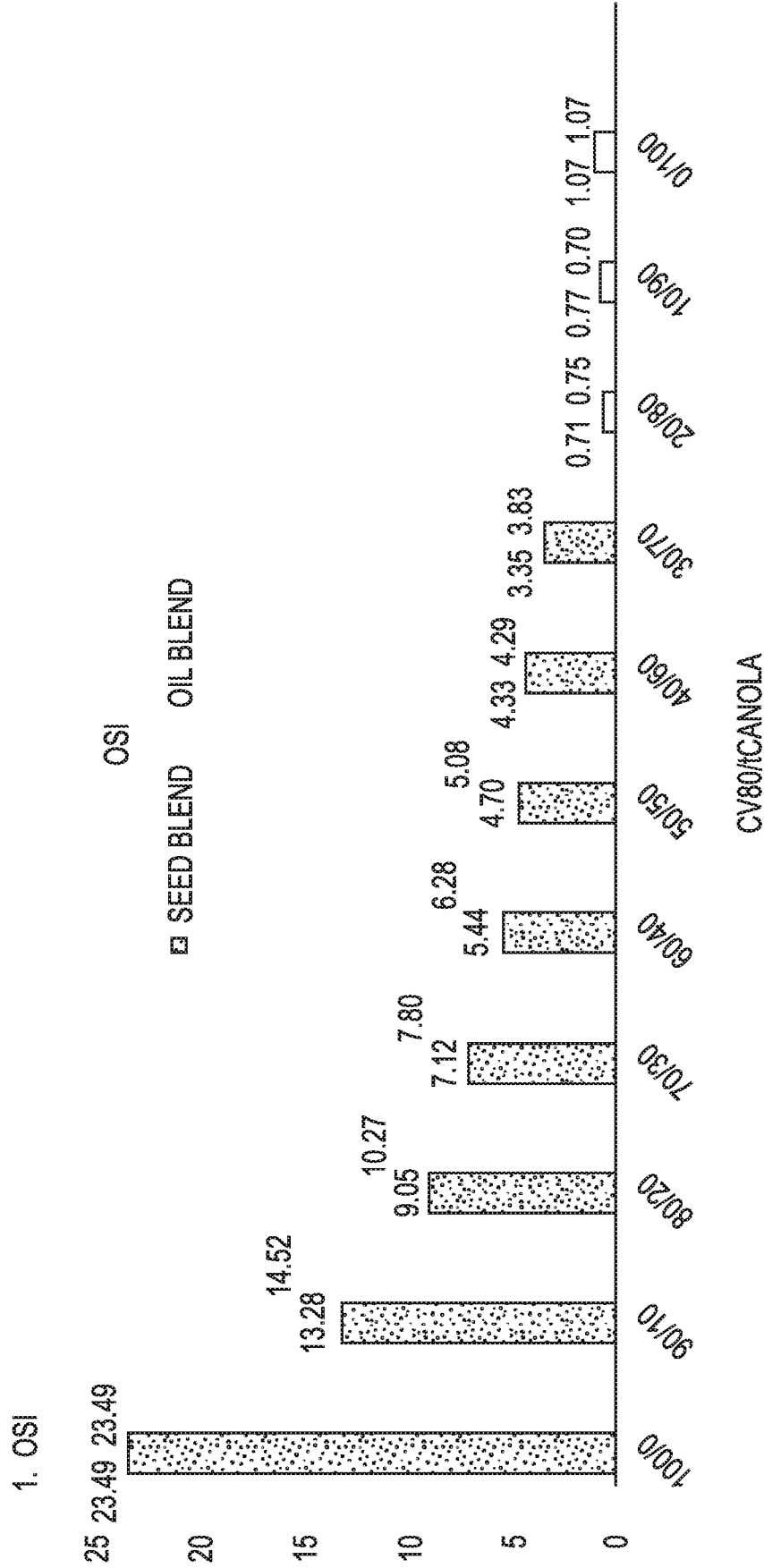


Figure 1

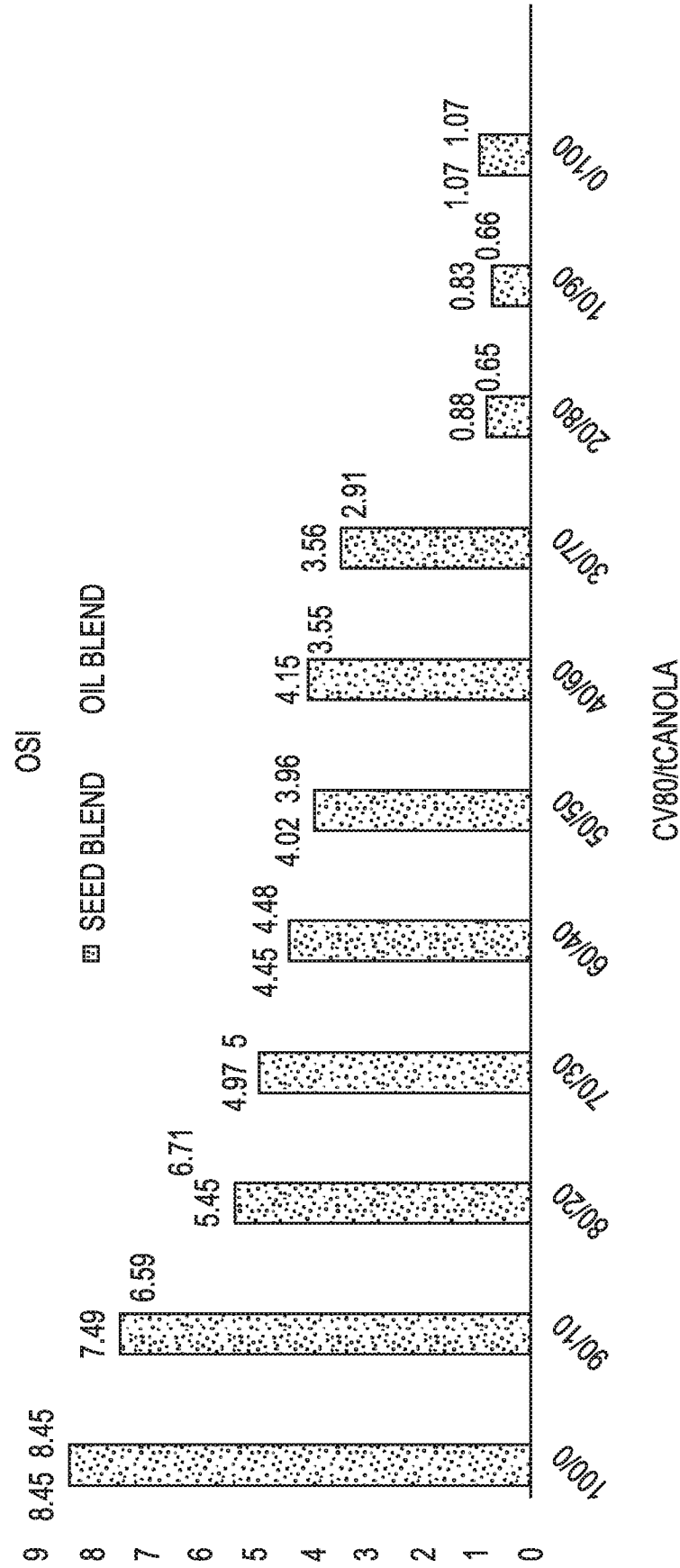


Figure 2

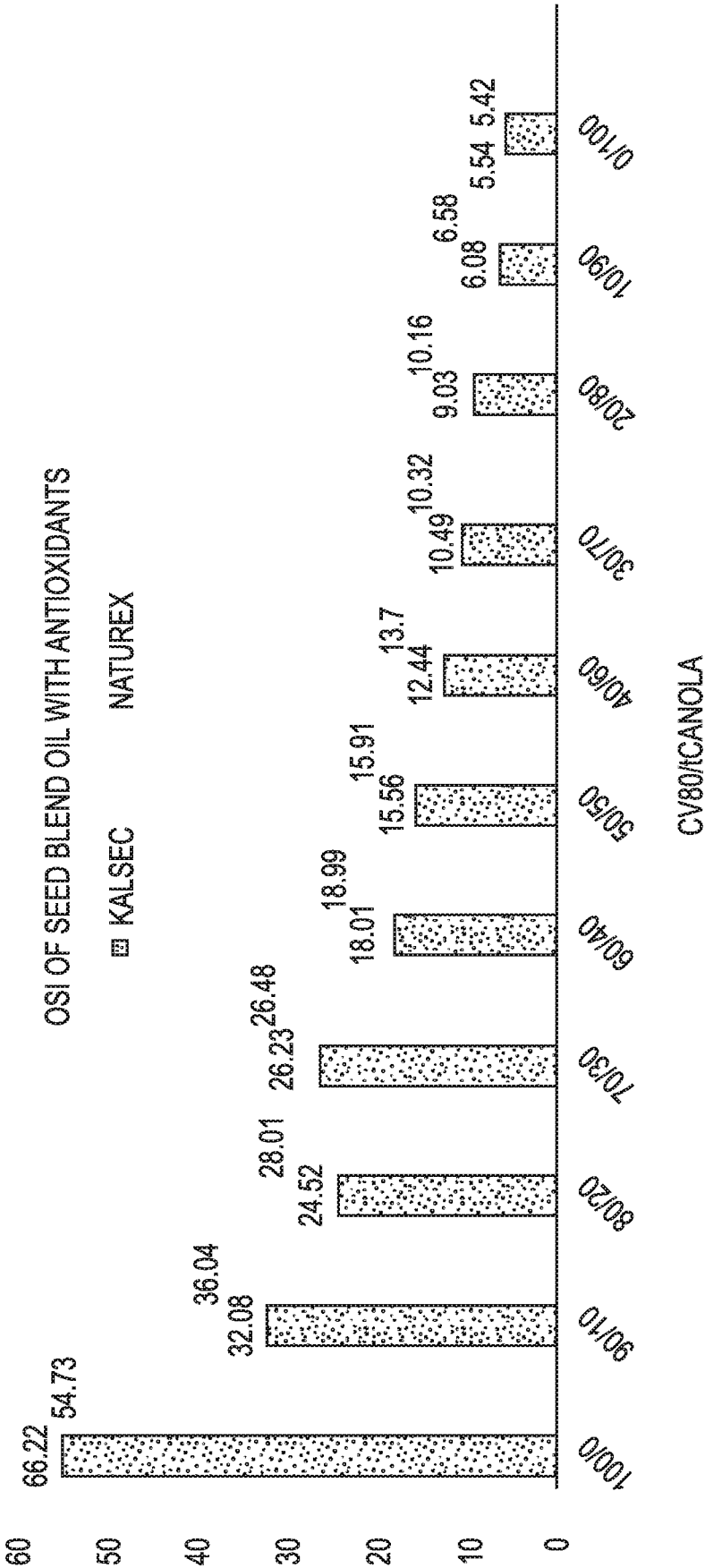


Figure 3

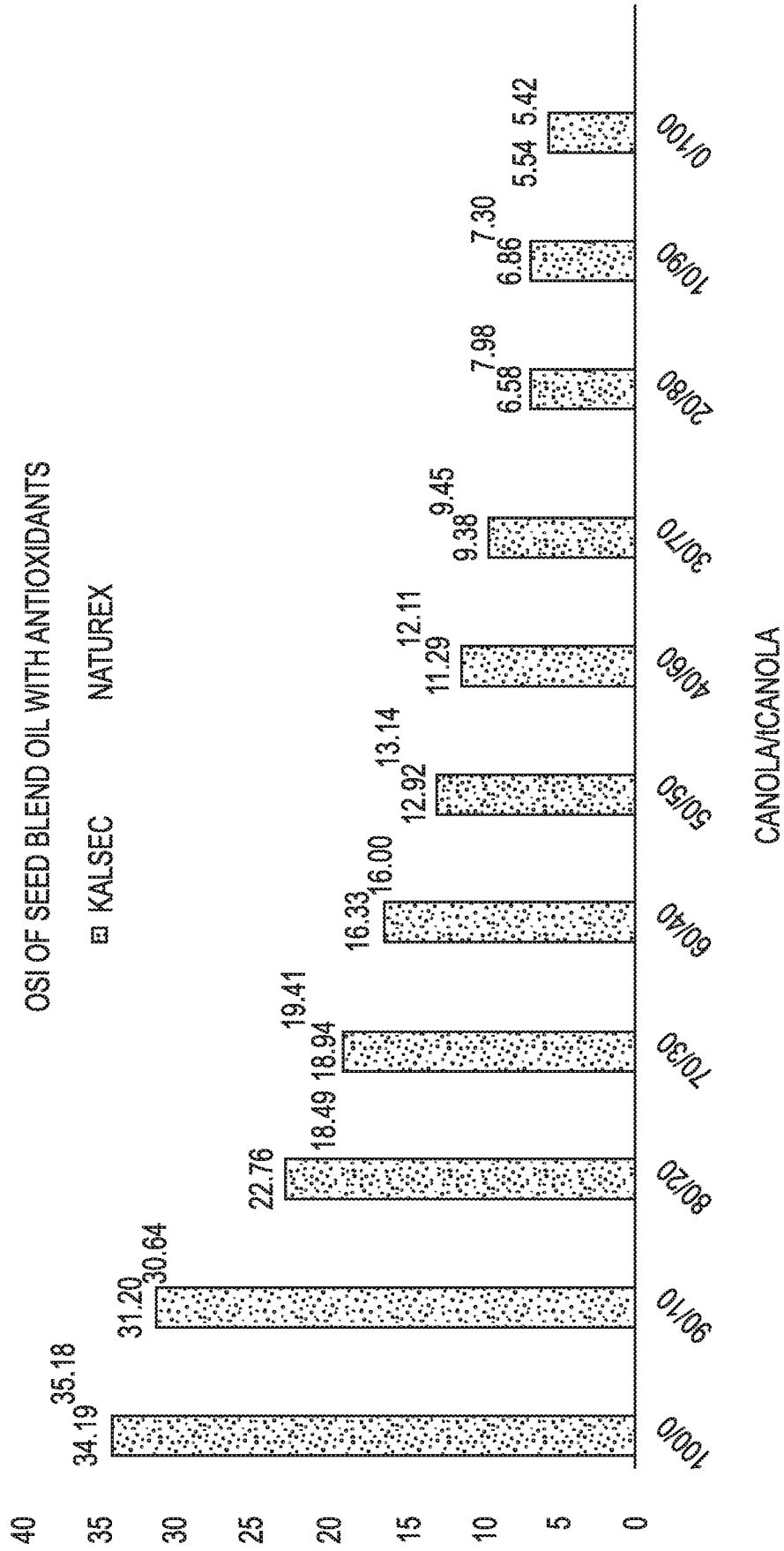
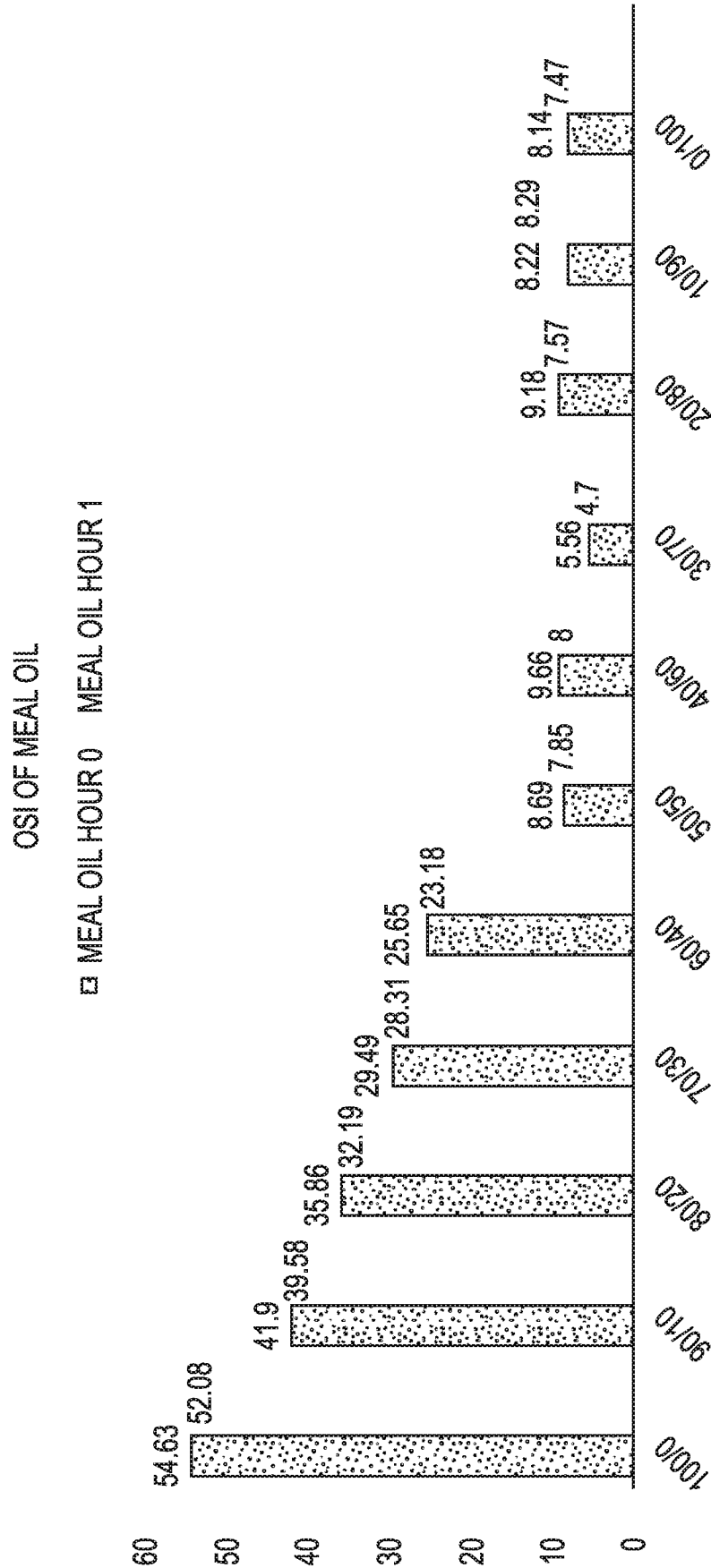


Figure 4



CV80/cANOLA

Figure 5

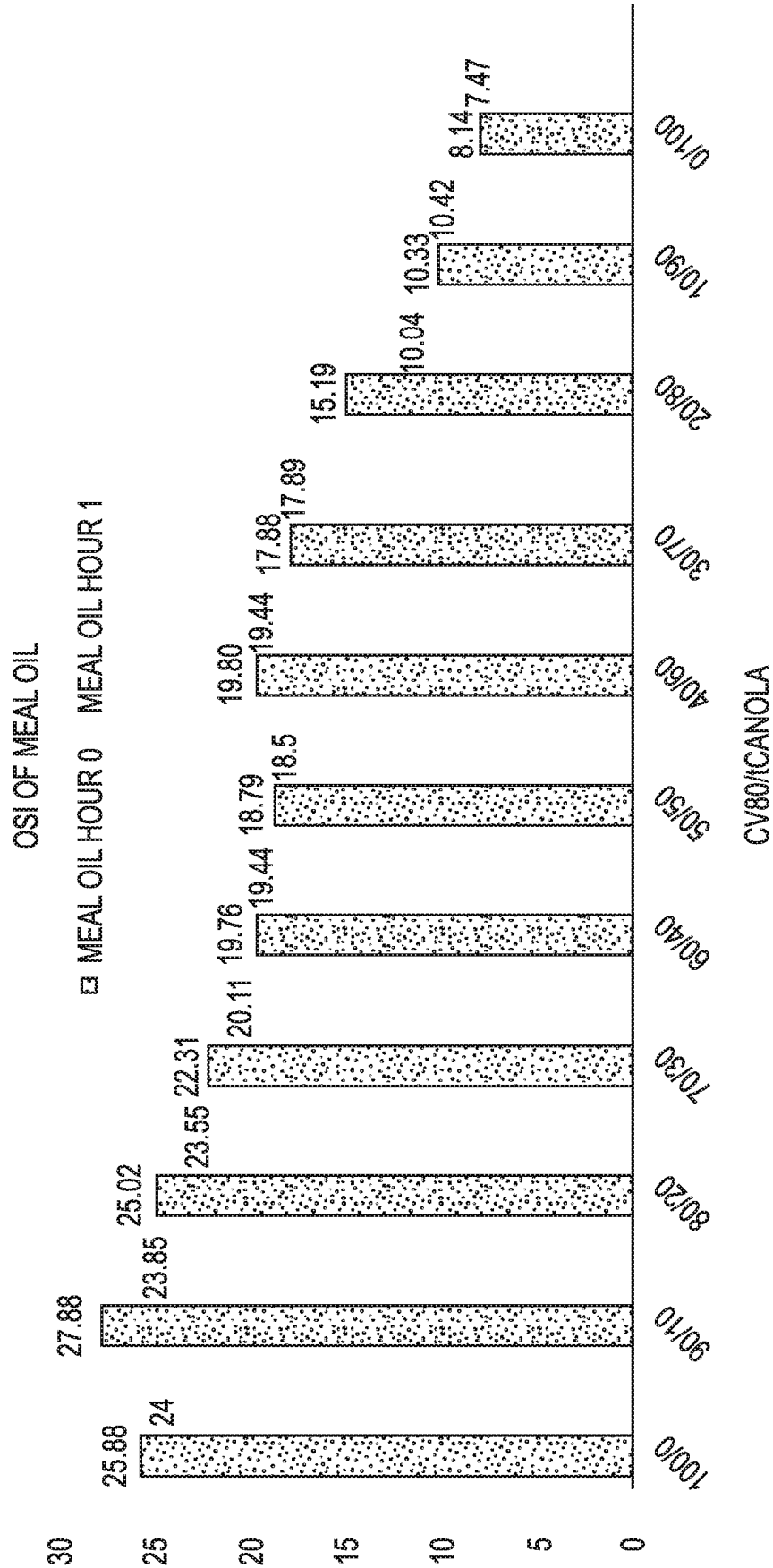


Figure 6

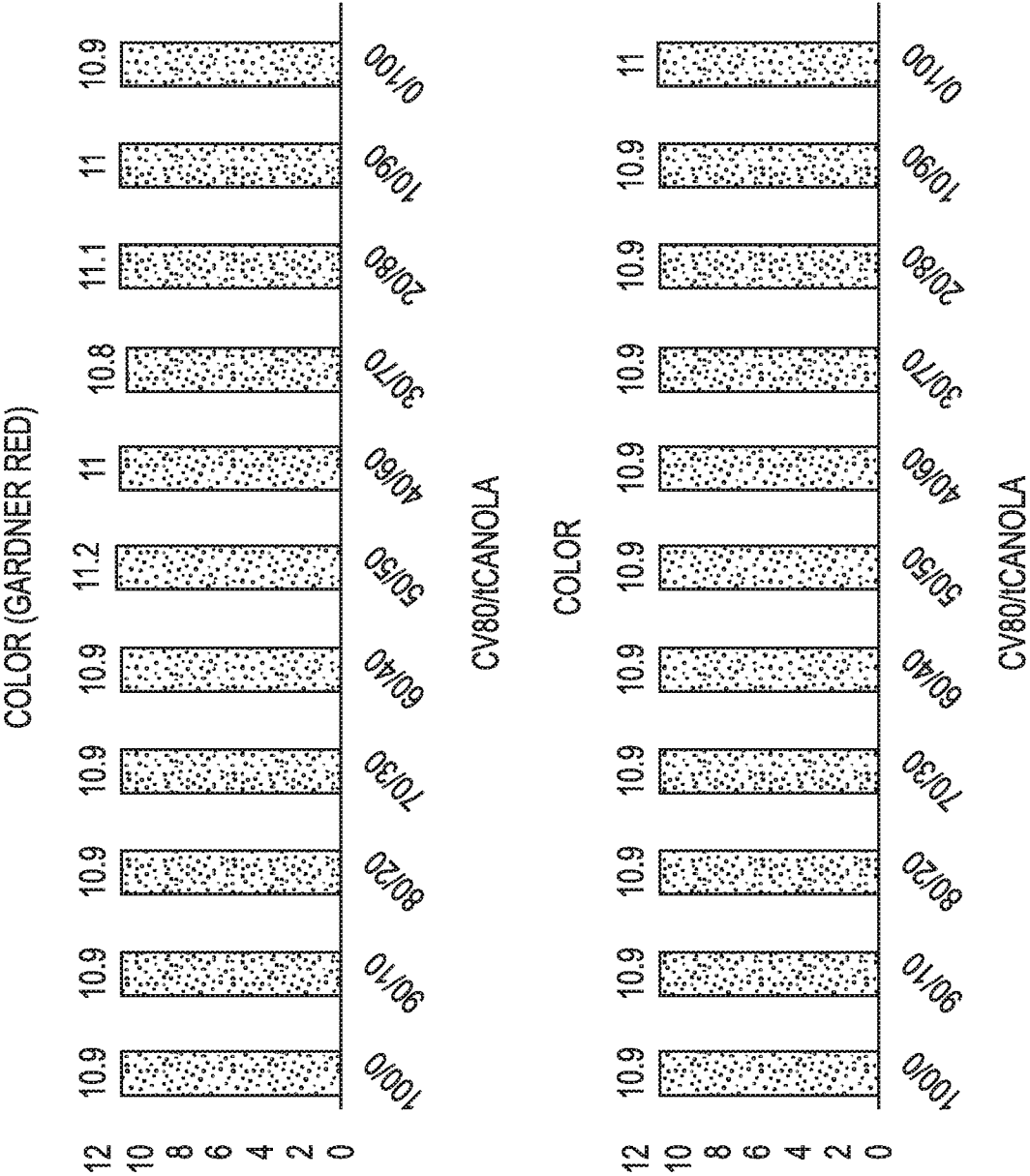


Figure 7

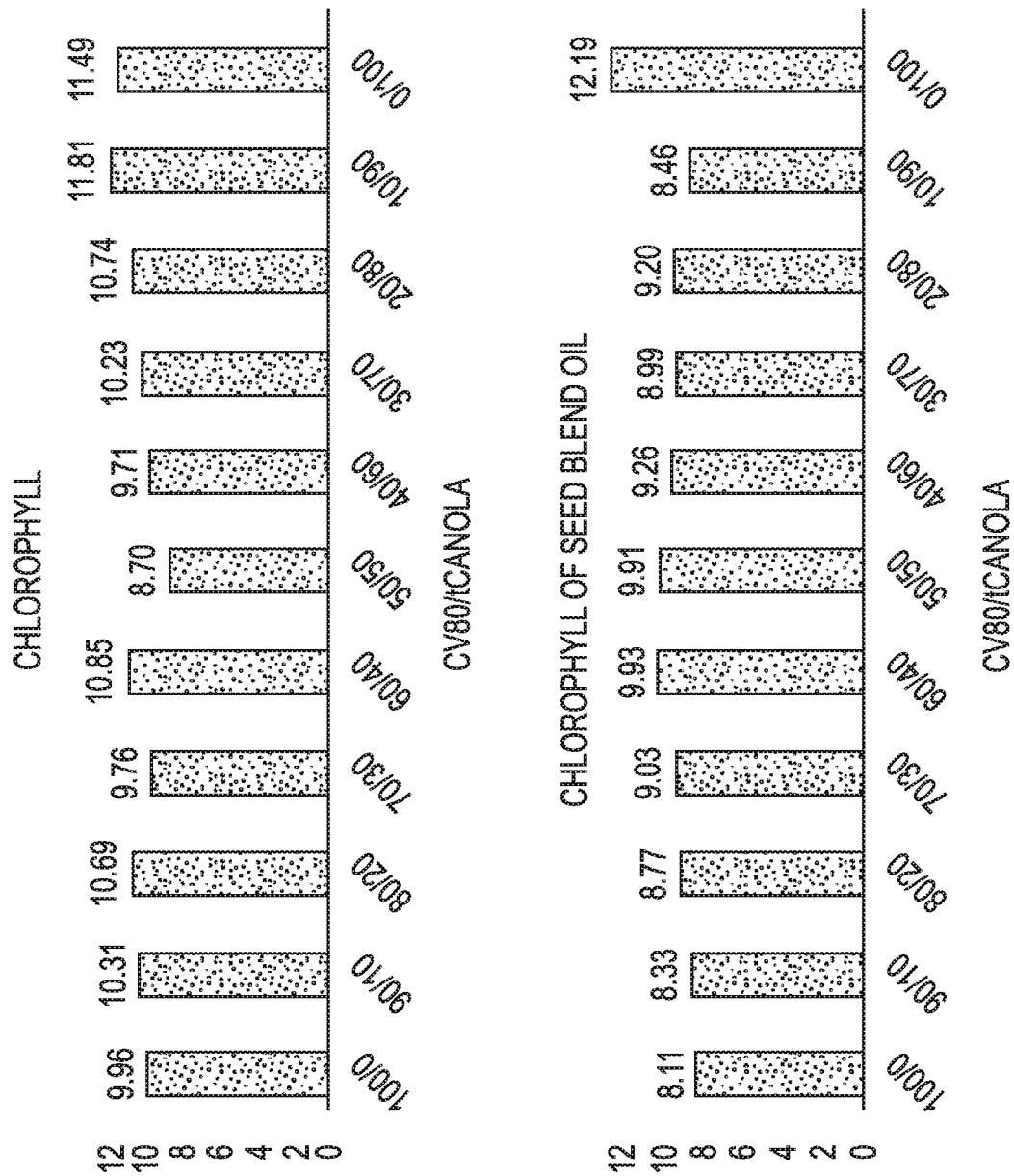


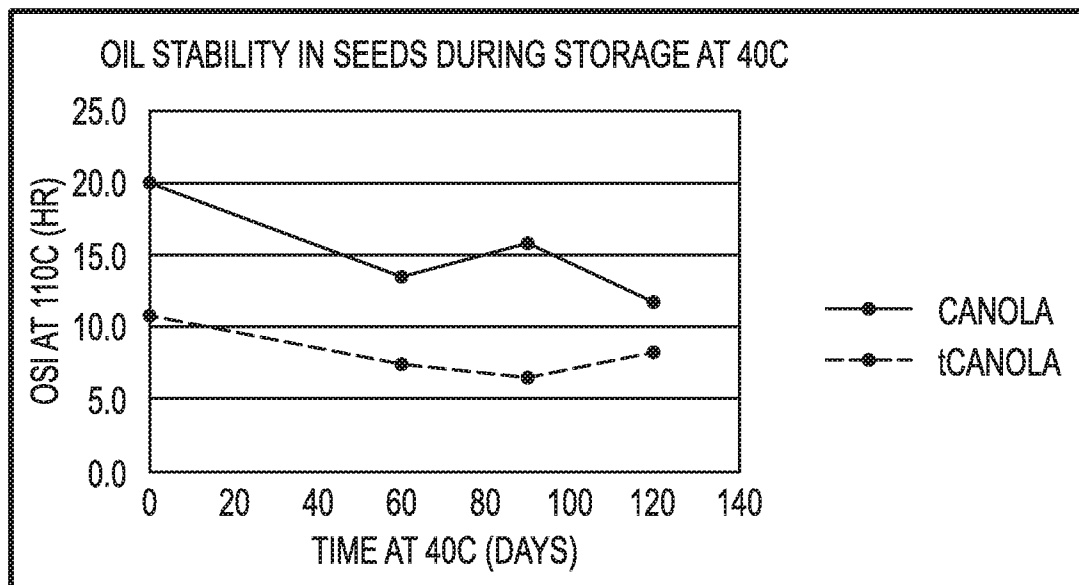
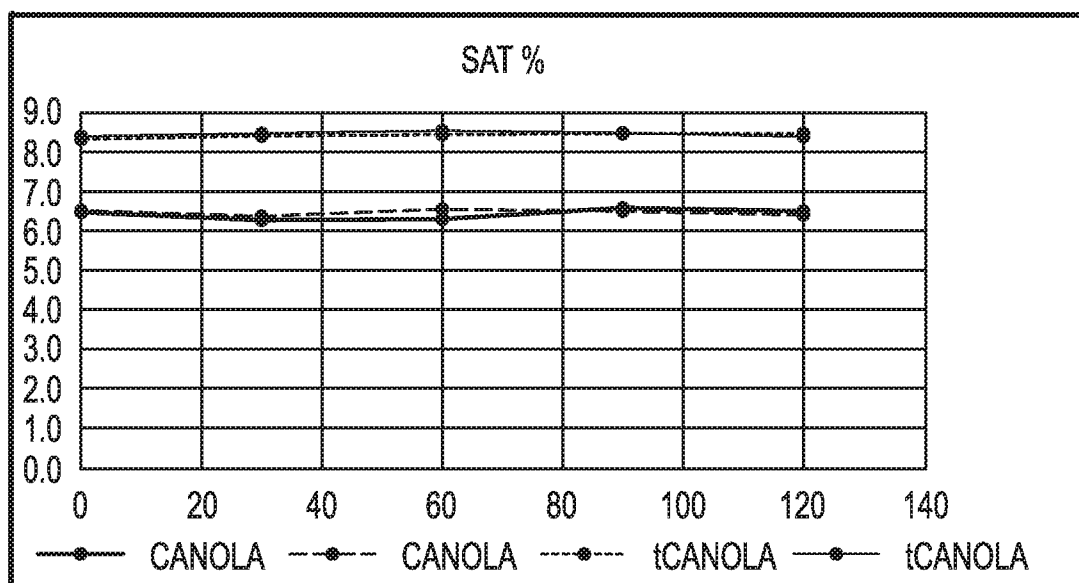
Figure 8

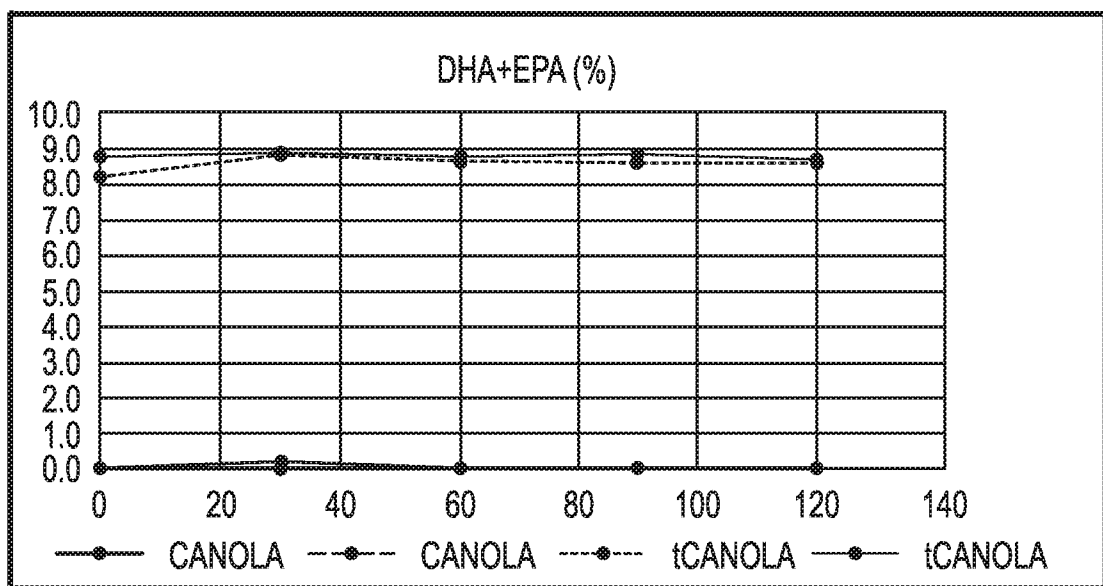
FATTY ACID PROFILE

CV80/ Dol	100:0		90:10		80:20		70:30		60:40		50:50		40:60		30:70		20:80		10:90		0:100	
	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB
C18:1	80.13	80.35	75.77	75.63	70.78	70.40	66.50	65.19	60.79	60.32	55.70	55.20	52.98	50.11	48.27	45.18	40.35	39.87	35.45	34.89	29.94	29.90
C18:2	8.16	8.15	10.05	10.33	12.29	12.48	14.11	14.69	16.77	16.77	18.79	18.96	20.04	21.14	21.88	23.26	25.24	25.47	27.58	27.68	29.87	29.84
C18:3	2.37	2.38	2.82	2.86	3.35	3.34	3.78	3.84	4.42	4.30	4.84	4.78	5.14	5.28	5.55	5.76	6.26	6.28	6.78	6.73	7.19	7.22
C18:5	0.00	0.00	0.66	0.75	1.39	1.49	2.02	2.26	2.96	2.95	3.60	3.72	4.02	4.47	4.68	5.17	5.86	5.90	6.51	6.77	7.35	7.36
C18:6	0.00	0.00	0.11	0.00	0.21	0.24	0.31	0.36	0.48	0.46	0.55	0.60	0.60	0.71	0.73	0.18	0.93	0.92	0.99	1.10	1.14	1.16
EPA+ DHA	0.00	0.00	0.77	0.75	1.60	1.73	2.33	2.62	3.43	3.41	4.15	4.32	4.62	5.18	5.41	5.97	6.79	6.82	7.50	7.87	8.50	8.52
SAT	6.91	6.86	7.06	6.97	7.28	7.32	7.56	7.60	7.60	7.84	7.92	7.98	8.09	8.25	8.25	8.45	8.63	8.68	8.78	8.77	9.08	9.08

CANOLA /DOL	100:0		90:10		80:20		70:30		60:40		50:50		40:60		30:70		20:80		10:90		0:100	
	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB	SB	OB
C18:1	61.30	61.30	59.31	58.35	54.33	55.43	51.66	52.29	49.05	49.11	45.99	45.85	45.60	42.64	41.52	39.36	34.54	35.91	33.32	33.07	29.90	29.90
C18:2	19.48	19.48	20.22	20.54	21.82	21.57	22.63	22.63	23.64	23.66	24.52	24.70	24.74	25.73	26.06	26.77	28.08	27.77	28.51	28.81	29.89	29.89
C18:3	9.80	9.80	9.59	9.57	9.22	9.30	8.96	9.03	8.84	8.78	8.55	8.54	8.50	8.30	8.15	8.02	7.56	7.79	7.44	7.53	7.17	7.17
C18:5	0.00	0.00	0.57	0.75	1.74	1.46	2.39	2.18	2.93	2.91	3.66	3.62	3.74	4.35	4.68	5.17	6.38	6.00	6.63	6.56	7.32	7.32
C18:6	0.00	0.00	0.10	0.13	0.28	0.23	0.40	0.34	0.48	0.45	0.61	0.57	0.61	0.67	0.76	0.82	1.07	0.97	1.11	1.02	1.11	1.11
EPA+ DHA	0.00	0.00	0.68	0.88	2.02	1.69	2.79	2.52	3.41	3.36	4.28	4.18	4.35	5.03	5.44	5.99	7.45	6.96	7.74	7.58	8.43	8.43
SAT	6.82	6.82	6.86	6.83	6.99	6.93	7.09	7.08	7.18	7.22	7.29	7.40	7.35	7.50	7.52	7.63	7.78	7.70	7.82	7.93	8.13	8.13

Figure 9

**Figure 10****Figure 11**

**Figure 12**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/44660

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C11B 1/00; C11B 1/06; C11B 1/08; C11B 1/10; C11B 5/00; C11C 1/00; A23D 9/02 (2016.01)

CPC - C11B 1/00; C11B 1/06; C11B 1/08; C11B 1/10; C11B 5/00; C11C 1/00; A23D 9/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C11B 1/00; C11B 1/06; C11B 1/08; C11B 1/10; C11B 5/00; C11C 1/00; A23D 9/02 (2016.01)

CPC - C11B 1/00; C11B 1/06; C11B 1/08; C11B 1/10; C11B 5/00; C11C 1/00; A23D 9/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 554/9; 554/224

Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Pub West (US EP JP WO), Pat Base (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), Google Patent, Google Scholar, Free Patents Online; search terms: canola, rapeseed, brassica, eicosapentanoic, docosahexaenoic, EPA, DHA, DPA, seed, oilseed, mix, premix, blend, combine, PUFA, omega-3, antioxidant, TBHQ, BHT, press

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	WO 2014/089274 A1 (CARGILL, INC.) 12 June 2014 (12.06.2014) para [0006], [0019]-[0022], [0026], [0037], [0054]	1-10, 13-14
Y	US 2008/0280009 A1 (GREITHER) 13 November 2008 (13.11.2008) para [0016], [0039]	1-10, 13-14
Y	US 2006/0110521 A1 (HEISE et al.) 25 May 2006 (25.05.2006) para [0081], [0083]	2-3
Y	US 2013/0177692 A1 (WENSING et al.) 11 July 2013 (11.07.2013) para [0056], [0057], [0134], [0155]	4-6, 9-10, 13-14
Y	GB 2147911 A (SOCIETE DES PRODUITS NESTLE S A) 22 May 1985 (22.05.1985) p 2, ln 27-30; p 3, ln 2-3	7-8
Y	US 5,696,278 A (SEGRS) 09 December 1997 (09.12.1997) the entire document	1-10, 13-14
Y	US 2007/0066565 A1 (KINCS et al.) 22 March 2007 (22.03.2007) the entire document	1-10, 13-14



Further documents are listed in the continuation of Box C.



* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 September 2016 (14.09.2016)

Date of mailing of the international search report

18 NOV 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/44660

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 11-12 and 15-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.