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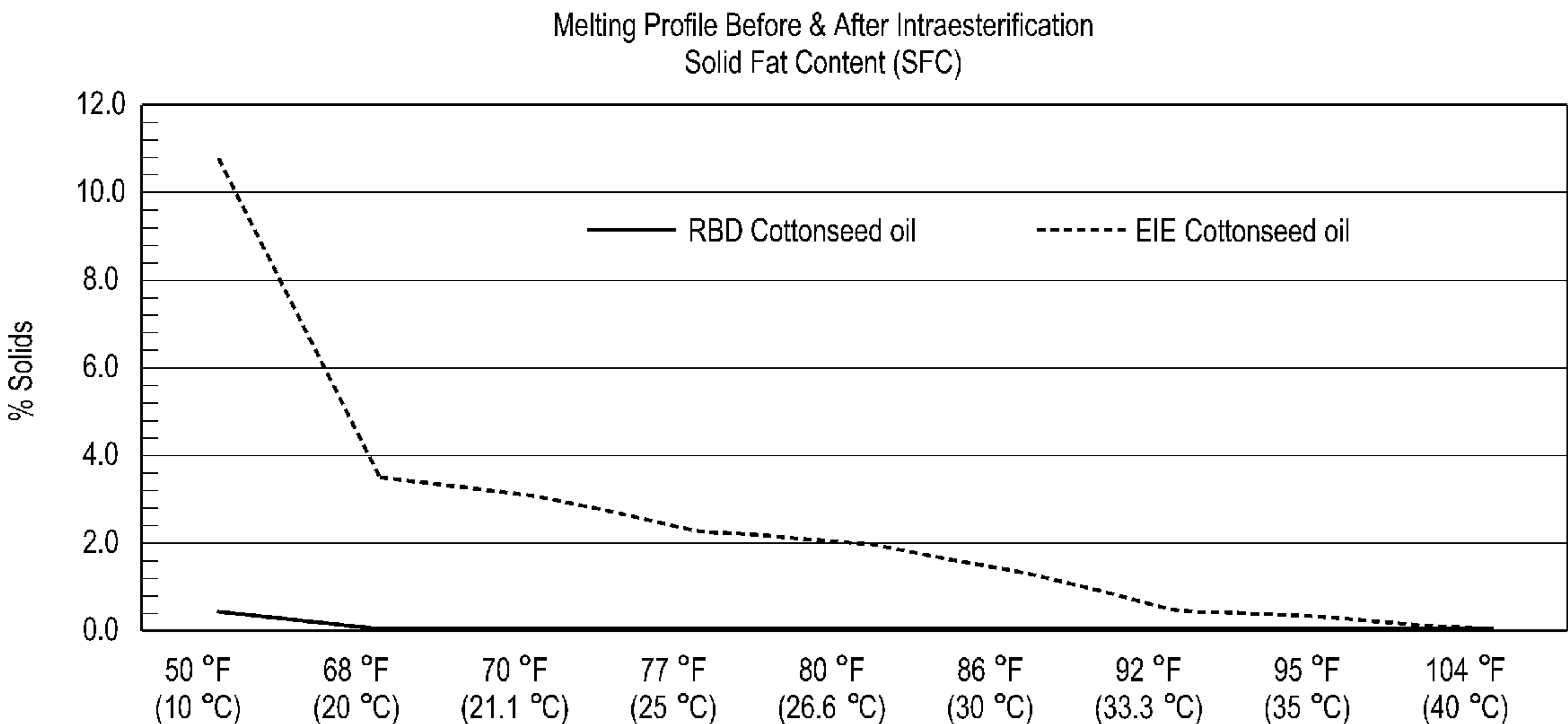


FIG. 1

(57) Abstract: The present invention relates to a composition comprising a 1,3-selective enzymatic intraesterified oil product having a greater unsaturated-saturated-unsaturated (USU) fatty acid content on the glycerol moiety than the oil from which it was derived. In an embodiment, the starting oil is cottonseed oil and in USU the U is primarily linoleic acid and the S is primarily palmitic acid. Further, the present invention relates to a method for increasing the USU content in an oil, comprising performing 1,3-selective enzymatic intraesterification of a regular starting oil wherein saturated-unsaturated-saturated (SUS) content is greater than the USU content in the starting oil prior to performing 1,3-selective enzymatic intraesterification. Due to the greater USU content, the 1,3-selective enzymatic intraesterified oil product has an increased melting temperature and solid fat content and therefore useful for margarines and food spreads as compared to the oil it was produced from.

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ENZYMATIC INTRAESTERIFICATION OF NON-TROPICAL PLANT OIL FOR STRUCTURING OF FOOD SPREADS AND MARGARINE

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FIELD OF THE INVENTION

[01] The present invention relates to an intraesterified non-tropical plant oil triglyceride product, such as an intraesterified cottonseed oil triglyceride product, having increased unsaturated-saturated-unsaturated content than in the non-intraesterified oil from which it was derived.

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BACKGROUND OF THE INVENTION

[02] Oils used for food spreads and margarine typically comprise tropical oils, such as palm oil or palm kernel oil, or comprise hydrogenated vegetable oils that are typically blended with other vegetable oils to achieve desired sensory and spreading properties. A disadvantage of tropical oils is that they are derived from forestry plants and are thus not deemed as sustainable as vegetable oils that are derived from annual plants. Another disadvantage of tropical oils is that they are derived from tropical forestry plants that do not commonly grow in non-tropical climates. Conventional processes for modifying natural vegetable oils for use in food products include hydrogenation and interesterification.

[03] Hydrogenation involves addition of hydrogen to convert unsaturated fatty acids to saturated fatty acids, thereby converting liquid vegetable oils to solids or semi-solids. The addition of hydrogen changes the degree of saturation of fat in the oil, thereby changing the melting range of the oil. The food industry has moved away from partially hydrogenated fats and towards fully hydrogenated fats, interesterified fats, and tropical oils (even though tropical oils have disadvantages as noted in the preceding paragraph).

[04] Interesterification involves blending of at least two different oils so that the fatty acids of these oils are redistributed among the triglycerides of these oils. Interesterification can occur in two ways, i.e., chemical interesterification or enzymatic interesterification. In chemical interesterification, a catalyst, e.g., sodium methoxide, is added to the oils and the triglyceride exchange is initiated with the aid of mild heat and agitation. Chemical interesterification results in random redistribution of fatty acids across glycerol backbones of the triglycerides to create new triglycerides with different solid fat content and melting behaviour. Enzymatic interesterification is a process of using an enzyme, e.g., a lipase, to rearrange the fatty acids of at least two different oils having varying triglycerides to create new triglycerides with different solid fat and melting behaviour.

[05] US 2015/0166932, discloses an intraesterification method in which the fatty acids of the triglycerides of a single high stearic high oleic (HSHO) oil, normally rich in saturated-unsaturated-unsaturated (SUU) type triglycerides, are randomly redistributed between the triglycerides to obtain a fat with an increased amount of saturated-unsaturated-saturated (SUS), saturated-saturated-unsaturated (SSU) type, and saturated-saturated-saturated (SSS) type triglycerides.

[06] US 9,795,152 discloses a 1,3-specific intraesterification method for increasing the SUS content in an oil that is normally rich in saturated-unsaturated-unsaturated (SUU) type triglycerides. The patent discloses that an important characteristic is the oxidative stability of the oil because the oxidation rate of linoleic acid (the main fatty acid in most of the liquid regular seedoils) is 40 times faster than oleic acid. The patent discloses triglycerides in which U (unsaturated fatty acid) is primarily L (linoleic acid) and accordingly commercial fats with this kind of triglyceride have a lower shelf life (or lower rancidity resistance) than those in which U is O (oleic acid). The patent therefore discloses that the starting oil or olein fraction is preferably selected from a high stearic high oleic (HSHO) oil. The patent discloses that the main characteristic in these types of oils is that U (unsaturated fatty acid) is primarily O (oleic acid), and this characteristic differentiates this type of oil from regular oils in which the main U is L (linoleic acid). As used herein, a “regular” oil is oil obtained from the most common natural cultivars of oil seed plants as opposed to being derived from special cultivars selected particularly for having an altered triglyceride content.

[07] Spread manufacturers desire base oils with sufficient solids to provide structuring from sources other than tropical oils, such as palm oil, palm oil fractions, interesterified palm oil/palm kernel blends or fully hydrogenated vegetable oil sources.

[08] It would be beneficial to have processes and products that do not have the disadvantages of conventional methods and products. For example, it would be beneficial to have an esterification process that uses a single regular or natural non-tropical plant oil as the feedstock to produce a triglyceride product having a melting temperature of at least 75° F. It would be beneficial to have a triglyceride product useful for margarines and food spreads, wherein the main U (unsaturated fatty acid) is L (linoleic acid), and wherein the triglyceride product has increased unsaturated-saturated-unsaturated content than in a regular or natural corresponding oil.

[09] It would be beneficial to have processes that do not have the disadvantages of conventional methods. For example, it would be beneficial to have a process that uses a single regular or natural non-tropical oil wherein the main U (unsaturated fatty acid) is L

(linoleic acid) to produce a fat with increased solid fat content useful for margarines and food spreads.

SUMMARY

[10] The present invention provides advantages over conventional methods and products.

5 In an aspect, a composition comprises a 1,3-selective enzymatic intraesterified oil product having a greater unsaturated-saturated-unsaturated (USU) content than the oil from which it was derived. As used herein, 1,3-selective enzymatic intraesterified oil product is a composition produced by enzymatic intraesterification of an oil having high saturated-unsaturated-saturated (SUS) content to convert that oil to an intraesterified oil product having
10 greater USU content than the oil from which is was derived by intra-rearrangement of fatty acids at the 1,3 position on the glycerol molecule under conditions that promote acyl migration of saturated fatty acids to the 2 position. In an embodiment, the U is primarily linoleic acid and the S is palmitic acid. In an embodiment, the 1,3-selective enzymatic intraesterified oil product is 1,3-selective enzymatic intraesterified cottonseed oil product. In
15 an embodiment, a 1,3 specific lipase enzyme (*e.g.*, *Thermomyces lanuginosis* (TLIM)) is used for the enzymatic Intraesterification. The 1,3 specific lipase enzyme promotes intra-rearrangement of fatty acids at the 1,3 position on the glycerol molecule. Also during lipase catalyzed re-arrangement, acyl migration to a varying degree will occur, exemplified primarily by migration of palmitic acid to the 2 position.

20 [11] An objective of the present invention is an intraesterified non-tropical triglyceride product having solid fat content (SFC) at 50° F that is greater than the basestock from which it was derived. For example, an objective of the present invention is an intraesterified non-tropical triglyceride product having solid fat content (SFC) at 50° F that is at least ten (10) times greater than the basestock from which it was derived.

25 [12] In an aspect, a composition comprises an intraesterified non-tropical triglyceride product having solid fat content (SFC) of at least 4% at 50°F, wherein the intraesterified non-tropical triglyceride product is derived from a non-tropical oil. In an aspect, a composition comprises an intraesterified non-tropical triglyceride product having solid fat content (SFC) of at least 7% at 50°F, wherein the intraesterified non-tropical triglyceride product is derived
30 from a non-tropical oil. In an aspect, a composition comprises an intraesterified non-tropical triglyceride product having solid fat content (SFC) of at least 9% at 50°F, wherein the intraesterified non-tropical triglyceride product is derived from a non-tropical oil. In an aspect, a composition comprises an intraesterified non-tropical triglyceride product having

solid fat content (SFC) of at least 9.5% at 50°F, wherein the intraesterified non-tropical triglyceride product is derived from a non-tropical oil.

[13] In an aspect, a composition comprises an intraesterified cottonseed triglyceride product having a melting temperature of at least 75°F.

5 [14] In an aspect, an intraesterified cottonseed triglyceride product has at least 25% more palmitic acid esterified to the second carbon of the glycerol greater than the basestock from which it was derived. In an aspect, the intraesterified cottonseed triglyceride product has at least 12% palmitic acid esterified to the second carbon of the glycerol.

10 [15] In an aspect, a vegetable oil spread comprises water, an emulsifying agent, and at least 5% by weight of a 1,3-selective enzymatic intraesterified oil product having a greater unsaturated-saturated-unsaturated (USU) content than the oil from which it was derived.

[16] In an aspect, a method comprises increasing the USU content in an oil by performing 1,3-selective enzymatic intraesterification of a natural starting oil wherein SUS content in the oil is greater than the USU content in the starting oil prior to performing 1,3-selective
15 enzymatic intraesterification. In a preferred embodiment, 1,3-selective enzymatic intraesterification is a continuous method.

[17] These and other aspects, embodiments, and associated advantages will become apparent from the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [18] FIG. 1 shows a melting profile (SFC) before and after enzymatic intraesterification (EIE) of cottonseed oil according to aspects of the present invention.

[19] FIG. 2 shows impact of residence time on solid fat content (SFC) according to aspects of the present invention.

[20] FIG. 3 shows flow rate change over first half-life of TLIM enzyme for a target
25 Mettler dropping point (“MDP”) of 82-83 degrees Fahrenheit with certain reaction conditions according to aspects of the present invention, including desired C16:0 sn-2 target.

[21] FIG. 4 shows a model graph for the texture analysis of a spread.

[22] FIG. 5 is a graph showing spread firmness as a function of ageing for spreads comprising intraesterified cottonseed triglyceride product in accordance with aspects of the
30 present invention as compared to a control spread comprising 75% soybean oil and 25% interesterified palm stearin/palm kernel oil, as characterized by peak force (g) versus time (weeks).

[23] FIG. 6 is a graph showing spread spreadability as a function of ageing for spreads comprising intraesterified cottonseed triglyceride product in accordance with aspects of the present invention as compared to a control spread comprising 75% soybean oil and 25% interesterified palm stearin/palm kernel oil, as characterized by peak force (g) versus time (weeks).

[24] FIG. 7 is a graph showing rate of crystallization at 50° F as characterized as SFC (% solids at 50° F) as a function of time for spreads comprising intraesterified cottonseed triglyceride product in accordance with aspects of the present invention as compared to a control spread comprising 75% soybean oil and 25% interesterified palm stearin/palm kernel oil.

[25] FIG. 8 is a graph showing rate of crystallization at 50° F as characterized as SFC (% solids at 50° F) as a function of time for spread basestocks comprising intraesterified cottonseed triglyceride product in accordance with aspects of the present invention, wherein the spread basestocks were deodorized and did not contain cotton stearin.

[26] FIG. 9 is a flow diagram depicting production of intraesterified cottonseed oil in accordance with aspects of the invention.

DETAILED DESCRIPTION

[27] The following acronyms and terms are used in this disclosure.

[28] EIE – Enzymatic Intraesterification. An esterification process that uses a single regular or natural oil and a lipase enzyme to rearrange fatty acids on the glycerol of triglyceride to create new triglycerides with different solid fat content (SFC) and melting behaviour than the single regular or natural oil.

[29] RB cottonseed oil – refined bleached cottonseed oil.

[30] RBD cottonseed oil – refined, bleached and deodorized cottonseed oil.

[31] SFC – solid fat content. The solid fat content is measured by NMR. The solid fat content method determines what percentage of all hydrogen nuclei (protons) in the test sample, composed of hydrogen nuclei in both liquid and solid phases, is due to hydrogen nuclei in the solid phase. This percentage is called the solid fat content.

[32] U – an unsaturated fatty acid (FA).

[33] S – a saturated fatty acid (FA).

[34] SUS – saturated-unsaturated-saturated type triglyceride.

[35] USU – unsaturated-saturated-unsaturated type triglyceride.

[36] MDP – Mettler dropping point. The dropping point of a fat or oil is the temperature at which the test sample will become fluid to flow under the conditions of the test. This is a temperature-related test in which a sample is heated at certain temperature rate until the material flows.

5 [37] IV – iodine value. The iodine value (or iodine adsorption value or iodine number or iodine index) in chemistry is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. The iodine value can also be calculated by fatty acid composition.

[38] GLC – gas liquid chromatography.

10 [39] FAC – Fatty acid composition. This measure determines the fatty acid present in an oil or fat.

[40] ROC – rate of crystallization (the rate at which a fat or oil crystalizes at a certain temperature as measured by SFC).

[41] Palmitic acid – a sixteen carbon containing fatty acid. Palmitic acid is a saturated
15 fatty acid.

[42] Stearic acid – an eighteen carbon containing fatty acid. Stearic acid is a saturated fatty acid.

[43] Oleic acid – an eighteen carbon containing fatty acid with one double bond. Oleic acid is an unsaturated fatty acid.

20 [44] Linoleic acid – an eighteen carbon containing fatty acid with two double bonds. Linoleic acid is an unsaturated fatty acid.

[45] Linolenic acid – an eighteen carbon containing fatty acid with three double bonds. Linoleic acid is an unsaturated fatty acid. Linolenic acid is an unsaturated fatty acid.

[46] Triglyceride – an ester formed from glycerol and three fatty acids attached to glycerol
25 through an ester linkage. The fatty acids are attached at the sn-1, sn-2, and sn-3 positions.

[47] sn-2 – the position of the fatty acid in the middle position or 2 position of the triglyceride.

[48] TLIM – the lipase enzyme known as *Thermomyces lanuginosis*.

[49] A process is disclosed herein for the production of a 1,3-selective enzymatic
30 intraesterified oil product having a greater unsaturated-saturated-unsaturated (USU) content than the starting oil from which it was derived or produced from. In an embodiment, the 1,3-selective enzymatic intraesterification is a continuous process. In an embodiment, the U is primarily linoleic acid and the S is palmitic acid. The 1,3-selective enzymatic intraesterified oil product may be used as a base oil for the production of water-in-oil spreads and

margarines, including but not limited to tub based water-in-oil food spreads and margarines. Those skilled in the art will recognize that margarine is no less than 80% fat or oil, whereas a food spread or other food application may have less than 80% fat or oil. The 1,3-selective enzymatic intraesterified oil product disclosed herein is derived from non-tropical oil and non-hydrogenated vegetable oil sources. The process and 1,3-selective enzymatic intraesterified oil product of the present invention have advantages over conventional methods and conventional oils derived from and tropical oils and hydrogenated vegetable oil sources.

[50] In an embodiment, the 1,3-selective enzymatic intraesterified oil product of the present invention is derived from cottonseed oil. Cottonseed oil does not have the same sustainability issues as tropical oils derived from tropical trees, such as palm oil. Cottonseed oil does not have the same health issues that have been raised by others in connection with use of partially hydrogenated vegetable oils.

[51] In an embodiment, the 1,3-selective enzymatic intraesterified oil product of the present invention has an increased level of solid fat content as compared to the oil from which it is derived.

[52] Those of ordinary skill in the art having the benefit of the present disclosure will recognize that the 1,3-selective enzymatic intraesterified oil product of the present invention may be used as a base oil in food spreads, margarines, panfrying products, and the like. The 1,3-selective enzymatic intraesterified oil products of the present invention may be used as an oil component in other food applications, including but not limited to meat alternatives, frozen food coatings, and frozen food novelty products.

[53] Turning to further description of the invention, in an aspect, a method comprises increasing the USU content in an oil by performing continuous 1,3-selective enzymatic intraesterification (EIE) of a natural starting oil wherein SUS content in the oil is greater than the USU content in the starting oil prior to performing 1,3-selective enzymatic intraesterification.

[54] In accordance with aspects of the invention, cottonseed oil has been enzymatically intraesterified to successively rearrange the starting material to produce a base oil with a solid fat content increase capable of providing enough solid fat for use in spreads or margarine.

[55] In accordance with aspects of the invention, a process comprises 1,3-selective enzymatic intraesterification using a lipase enzyme. In an embodiment, the lipase enzyme comprises *Thermomyces lanuginosis* (TLIM).

[56] In accordance with aspects of the invention, intraesterified cottonseed oil was produced utilizing a bench top intrasterification process in which cottonseed oil was passed through a column containing an immobilized 1,3 specific lipase enzyme (TLIM in this example) that promotes intra-rearrangement of fatty acids at the 1,3 position on the glycerol molecule. Also during lipase re-arrangement, acyl migration to a varying degree does occur, as exemplified by the migration of palmitic acid to the sn-2 position. More specifically, intraesterified cottonseed oil was produced utilizing a bench top process by placing 267 grams of TLIM enzyme (manufactured by Novozymes A/S, Denmark) in a heated water jacketed glass chromatography column that served as a fixed bed reactor. The lipase enzyme was slurried with cottonseed oil to aid in the adding of the enzyme to the fixed bed reactor. Cottonseed oil was then added to a reservoir and heated to 70°C. The cottonseed oil was then pumped with the utilization of a piston pump to the top of the fixed bed reactor of lipase enzyme at a designated flow rate of 727 grams per hour to pass the cottonseed oil through the column bed containing the enzyme. The column of enzyme was maintained at 70°C with the aid of a heated water jacket. As the cottonseed oil came into contact with the lipase enzyme, intra-rearrangement of the cottonseed oil fatty acids occurred. With the rearrangement of the cottonseed oil, new triglycerides were produced, thus modifying the physical properties of cottonseed oil to yield an increase in the solid fat content (SFC), melting point and palmitic acid in the sn-2 position. The cottonseed oil was continuously pumped through the fixed bed reactor to yield the amount of intraesterified cottonseed oil for characterization and small scale spread production using a scraped surface heat exchanger (manufactured by Armfield Limited, United Kingdom).

[57] FIG. 9 is a process flow diagram depicting intraesterification process 900 for production of intraesterified cottonseed oil in accordance with aspects of the invention. As shown in FIG. 9, intraesterification process 900 comprises conveying cottonseed oil 902 to a fixed bed reactor 904 comprising a 1,3 selective lipase enzyme, *e.g.*, TLIM enzyme. Cottonseed oil 902 may be conveyed by pump 906 from cottonseed oil feed source 908, *e.g.*, a cottonseed oil reservoir or feed tank, to fixed bed reactor 904. As shown in FIG. 9, cottonseed oil may be held or maintained in cottonseed oil feed source 908 at a temperature greater than ambient temperature, *e.g.*, 70°C. Cottonseed oil 902 may enter fixed bed reactor 904 through inlet port 910 of fixed bed reactor 904. Inlet port 910 may be located at the top of fixed bed reactor 904. The flow rate of cottonseed oil 902 conveyed to fixed bed reactor 904 may be at a predetermined flow rate, *e.g.*, 727 grams/hour. The amount of enzyme in fixed bed reactor 904 may be a predetermined amount, *e.g.*, 267 grams of enzyme. The ratio

of the flow rate of cottonseed oil 902 conveyed to fixed bed reactor 904 to the amount of enzyme in fixed bed reactor 904 may be a predetermined ratio, .e.g., 727 grams/hour of cottonseed oil 902 to 267 grams of enzyme, *i.e.*, 2.72. The temperature in fixed bed reactor 904 may be maintained at a temperature greater than ambient temperature, *e.g.*, 70°C. In
5 fixed bed reactor 904, cottonseed oil 902 is converted to intraesterified cottonseed oil 912. Intraesterified cottonseed oil 912 may exit fixed bed reactor 904 through outlet port 914 of fixed bed reactor 904. Outlet port 914 may be located at the bottom of fixed bed reactor 904. Intraesterified cottonseed oil 912 may be conveyed from fixed bed reactor 904 to intraesterified cottonseed oil product tank 916.

10 [58] Crystallization trials were run to produce a 60% fat based spread. Two different lots of enzymatically intraesterified cottonseed oil were successful in producing a stable under-refrigeration 60% fat based spread. Water in oil emulsions were produced in the following manner. Intraesterified cottonseed oil was added to a stainless steel container and heated to 60°C. Distilled monoglycerides and lecithin were then added to assist in water in oil
15 emulsification. Water was added to a separate stainless steel container and warmed, and salt was added to the water and stirred until dissolved in the water. The water with dissolved salt was then slowly added to the intraesterified cottonseed oil containing the emulsifiers. The mixture was then heated and agitated to ensure homogeneity. The water in oil emulsion was then passed through a scraped surface heat exchanger (manufactured by Armfield Limited,
20 United Kingdom) to initiate crystallization. The emulsion was then passed through two scraped surface heat exchange barrels and one pinworking barrel to produce a homogeneous water in oil 60% fat spread. The two scraped surface heat exchangers barrels were chilled to between 4°C – 10°C to initiate lipid crystallization, whereas the pinworking barrel was not cooled, to allow for crystal growth and even distribution of lipid crystal. The use of a
25 scraped surface heat exchanger is a common practice to evaluate a variety of basestocks that can be used in water in oil spread formulations.

[59] Table 1 provides an analysis of basestock cottonseed oil and enzymatically intraesterified (EIE) cottonseed oil derived from the basestock cottonseed oil. As shown in Table 1, intraesterified RBD cottonseed oil had 10.72 % SFC at 50° C, whereas the basestock
30 RBD cottonseed oil from which it was derived had 0.43% SFC at 50° C.

[60] As shown in Table 1, intraesterified RBD cottonseed oil had 2-monopalmitin (*i.e.*, palmitic acid linked to the 2 position of glycerol) at 19.35% by weight, whereas the basestock RBD cottonseed oil from which it was derived had 2-monopalmitin at 2.46% by

weight. Thus, the oil was converted from an oil that was SUS to an oil that was USU by the 1,3 selective lipase.

[61] Table 1 shows the FAC profile of intraesterified RBD cottonseed oil and the basestock RBD cottonseed oil from which it was derived is set forth in the row beginning
5 with “Myristic C14:0” through “Total Saturated FAs.”

[62] FIG. 1 is a graph of data set forth in Table 1, depicting the melting profile before and after enzymatic intraesterification (EIE) of RBD cottonseed oil, as characterized by % solids versus temperature.

Table 1

Analysis	Basestock Before Intraesterification RBD Cottonseed Oil (CSO) 5995-67	Intraesterified RBD Cottonseed Oil (CSO) 5995-67
Mettler Drop Point (F)	51.44	82.40
Iodine Value (IV) by GLC	110.2	110.4
SFC @ 50°F (10C)	0.43	10.72
SFC @ 68°F (20C)	0.00	3.49
SFC @ 70°F	0.00	3.07
SFC @ 77°F (25C)	0.00	2.30
SFC @ 80°F	0.00	2.01
SFC @ 86°F (30C)	0.00	1.38
SFC @ 92° F	0.00	0.49
SFC @ 95°F (35C)	0.00	0.30
SFC @ 104°F (40C)	0.00	0.00
Lovibond Color (Y)		34.00
Lovibond Color (R)		2.00
Diacylglycerol (DAG)	2.35	3.66
2-MonoMyristin	0.54	1.11
2-MonoPalmitin	2.46	19.35
2-MonoStearin	0.32	1.97
2-MonoOlein	19.92	17.11
2-MonoLinolenin	75.47	60.15
2-MonoArachidin	0.12	0.22
Myristic C14:0	0.81	0.81
Palmitic C16:0	22.99	22.94
Palmitoleic C16:1	0.55	0.56
Stearic C18:0	2.56	2.55
Total C18:1 trans FA	0.23	0.12
Total C18:1 cis FA	16.99	17.09
Total C18:2 trans FA	0.78	0.96
Total C18 2 cis FA	53.55	53.40
Total C18:3 trans FA	0.03	0.05
Total C18:3 cis FA	0.24	0.26
Arachidic C20:0	0.29	0.28
Linolenic C18:3n3cis	0.19	0.18
Behenic C22:0	0.14	0.14
Lignoceric C24:0	0.08	0.09
Total trans FA	1.05	1.13
Total Saturated FAs	27.02	26.96

[64] Table 2 provides the formula for a 60% fat spread with EIE cottonseed oil. 90% Distilled Alpha Mono and soy lecithin are emulsifiers. In this example, the 90% distilled alpha mono was Alphadim 90 SBK, and the soy lecithin was Yelkin SS. Natural beta

carotene was included in the fat spread formula to provide yellow coloring so that the spread had color more similar to butter than without the addition of the natural beta carotene.

Table 2 – 60% Fat Spread Formula

Fat Phase % by weight	Water Phase % by weight
59.2990% Intraesterified Cottonseed Oil	38.7% Water
0.5000% 90% Distilled Alpha Mono	1.3000% Salt
0.2000% Soy Lecithin	
0.0010% Natural Beta Carotene	
60% Total	40% Total

5 [65] Those skilled in the art, having the benefit of this disclosure, will recognize the attributes of the base stock and the above 60% fat spread with EIE cottonseed oil derived therefrom: (i) no palm or other tropical oil; (ii) no hydrogenation; (iii) moderate in saturates (around 25-28% saturates versus around 50% saturates in palm and other tropical oils); and (iv) good source of linoleic oil, an omega-6 essential fat.

10 [66] Table 3 provides an analysis of basestock cottonseed oil and enzymatically intraesterified (EIE) cottonseed oil derived from the basestock cottonseed oil. As shown in Table 3, the cottonseed oil basestock had a SFC of 0.32% at 50° F and 2-monopalmitin of only 2.10% in the 2-position of the triglyceride, whereas EIE undeodorized cottonseed oil produced on different collection dates 1 through 8 (i.e., “Collect 1, Collect 2, etc.”) from the
 15 basestock in accordance with aspects of the present invention had SFC at 50° F ranging from 10.93% (Collect 2) to 9.63% (Collect 7) and 2-monopalmitin in the 2-position of the triglyceride ranging from 18.68% (Collect 1) to 17.66% (Collect 5). When EIE undeodorized cottonseed oil produced on the different collection dates 1 through 8 were combined in equal amounts as an “all eight runs homogeneous” sample, that “all eight runs
 20 homogeneous” sample had SFC of 10.98% at 50° F and 2-monopalmitin in the 2-position of the triglyceride of 18.00%. When the “all eight runs homogenous” sample of EIE undeodorized cottonseed oil produced on different collection dates was then deodorized, the

deodorized all collects sample had SFC of 10.23% at 50° F and 2-monopalmitin in the 2-position of the triglyceride of 17.90%.

Table 3

Analytical	Basestock RBD CSO Lot# 012218D 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 1 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 2 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 3 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 4 Homog 5995-98	Intraesterified CSO Undeodorized Collect 5 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 6 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 7 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench Collect 8 Homog 5995-98	Intraesterified CSO Undeodorized Large Bench All Eight Runs Homogenous 5995-98	Intraesterified CSO Deodorized Large Bench All Eight Runs Homogenous 5995-98
Mettler Drop Point (F)	48.92	83.48	81.50	83.66	80.24	81.50	82.76	81.32	82.94	82.04	81.14
IV by GLC	112.1									112.3	111.6
SFC @ 50° F (10C)	0.32	10.46	10.93	10.46	10.04	10.11	10.22	9.63	10.02	10.98	10.23
SFC @ 68° F (20C)	0.13	3.16	3.54	3.19	3.46	3.09	3.28	3.16	3.05	3.21	3.22
SFC @ 70° F	0.10	3.01	3.06	3.04	2.93	2.66	2.93	2.83	2.76	2.93	2.93
SFC @ 77° F (25C)	0.00	1.87	2.20	2.06	2.13	2.33	2.28	1.90	2.16	2.11	2.15
SFC @ 80° F	0.00	1.69	1.68	1.73	1.57	1.51	2.02	1.49	1.78	1.86	1.83
SFC @ 86° F (30C)	0.00	1.38	1.33	1.08	1.18	1.10	1.28	1.02	1.17	1.20	1.28
SFC @ 92° F	0.00	0.75	0.79	0.82	0.50	0.43	0.46	0.57	0.82	0.81	0.72
SFC @ 95° F (35C)	0.00	0.37	0.27	0.05	0.17	0.15	0.11	0.12	0.38	0.18	0.31
SFC @ 104°F (40C)	0.00	0.08	0.04	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
2-MonoMyristin	0.00	1.27	1.35	1.67	1.44	1.52	1.92	1.32	1.38	1.27	1.54
2-MonoPalmitin	2.10	18.68	18.19	18.13	17.87	17.66	17.76	18.30	17.88	18.00	17.90
2-MonoStearin	1.90	0.00	2.11	2.02	1.74	1.25	1.72	1.24	1.35	2.01	1.53
2-MonoOlein	18.05	18.38	16.12	16.10	16.54	16.91	16.51	16.97	16.91	16.27	16.72
2-MonoLinolein	77.01	60.97	61.51	61.19	61.48	61.77	61.66	61.34	61.74	61.79	61.60
2-MonoLinolenin	0.76	0.37	0.40	0.52	0.47	0.53	0.44	0.47	0.51	0.47	0.53
2-MonoArachidin	0.18	0.33	0.33	0.37	0.36	0.35	0.00	0.36	0.23	0.19	0.20
Myristic C14:0	0.81	0.81							0.80	0.80	0.81
Palmitic C16:0	22.39	22.25							22.30	22.33	22.32
Palmitoleic C16:1	0.56	0.56							0.57	0.57	0.56
Stearic C18:0	2.39	2.37							2.37	2.39	2.55
Total C18:1 trans FA	0.29	0.31							0.29	0.28	0.19
Total C18:1 cis FA	16.48	16.52							16.50	16.48	16.80
Total C18:2 trans FA	0.69	0.68							0.67	0.70	0.85
Total C18 2 cis FA	55.11	55.18							55.07	55.13	54.47
Total C18:3 trans FA	0.00	0.01							0.00	0.01	0.02
Total C18:3cis FA	0.18	0.22							0.25	0.22	0.22
Arachidic C20:0	0.26	0.27							0.27	0.28	0.28
Gadoleic C20:1n9 cis	0.07	0.07							0.07	0.08	0.07
Behenic C22:0	0.12	0.13							0.14	0.13	0.15
Lignoceric C24:0	0.07	0.09							0.08	0.09	0.07
Total trans FA	0.98	1.00							0.96	0.98	1.06
Total Saturated FAs	26.17	26.05							26.12	26.15	26.35

[67] Table 4 shows a residence time study depicting the impact of residence time on SFC and C16:0 sn-@. The designation 1 X Decrease Residence means the residence time of the EIE cottonseed oil in the vessel was reduced in half (50%) from the regular residence time by reducing the amount of enzyme in the vessel by half (50%). The designation 2 X Decrease Residence means the residence time of the EIE cottonseed oil in the vessel was reduced in half again from 1 X Decrease Residence so the residence time was 25% of the regular residence time by reducing the amount of enzyme in the vessel by half again (25% enzyme compared to 100% enzyme used for the regular residence time). The residence time may also be referred to as retention time. With respect to Table 4, the regular residence time was 30.08 minutes, the 1 X Decrease residence time was 15.03 minutes, and the 2 X Decrease Residence Time was 7.49 minutes. These same residence times are identified in Table 5, discussed below. As shown in Table 4, reducing the residence time by 50% by reducing the amount of enzyme in the vessel by 50% resulted in a reduction of 16:0 sn-2, i.e., palmitic acid, fatty acid in the 2-position of the triglyceride from 19.35% to 12.29%. This value of 12.29% is greater than the 2.46% (see Tables 1 and 4, row for 2-MonoPalmitin) or 2.10% (see Table 3, row for 2-MonoPalmitin) for basestock RBD cottonseed oil (CSO). An objective of the present invention is an EIE product having an SFC at 50° F greater than the basestock the EIE product was derived from. An objective of the present invention is an EIE product having an SFC at 50° F at least ten (10) times greater than the basestock the EIE product was derived from.

[68] FIG. 2 is a graph of the data set forth in Table 4, showing the impact of residence time on SFC.

[69] In an embodiment, an EIE product has an SFC at 50° F of at least 4.0% at 50° F for spreadability functionality. As shown in Table 4, a 2 X Decrease Residence Time (for Collection Date 1), i.e., only 25% residence time/25% enzyme than regular residence time/enzyme amount, resulted in an SFC of 4.39% at 50° F. The basestock from which this EIE product was derived had an SFC of 0.32% (see Table 3). Thus, in this embodiment of the present invention, the EIE product had 13.7 times greater SFC than the basestock from which it was derived.

[70] In a preferred embodiment, the EIE of the present invention has an SFC of at least 9.0% at 50° F. In a more preferred embodiment, the EIE of the present invention has an SFC of at least 9.50% at 50° F. As shown in Table 4, a 1 X Decrease Residence Time (for Collection Date 1) resulted in an SFC of 10.45% at 50° F. A 50% residence time, i.e., reducing the amount of enzyme in the vessel by 50%, surprisingly provides an EIE

cottonseed oil having excellent spreadability functionality and is not much less than the SFC of 10.72% at 50° F for an EIE cottonseed oil under regular residence time, i.e., a regular amount of enzyme. The above SFC of 10.45% (1 X Decrease Residence Time) and 10.72% (regular residence time) at 50° F for EIE cottonseed oil is relatively close to SFC of about 12-15% at 50° F for interesterified blend of palm oil and palm kernel blends commonly used in the margarine and spreads industry.

Table 4

Analysis	Cottonseed Oil Base Stock	Regular Residence	Regular Residence	1 X Decrease Residence Time	2X Decreased Residence Time
SFC @ 50 F	0.43	10.72	11.97	10.45	4.39
SFC @ 68 F	0.00	3.49	4.19	3	1.62
SFC @ 70 F	0.00	3.07	3.78	2.7	1.55
SFC @ 77 F	0.00	2.3	2.6	1.78	1
SFC @ 80 F	0.00	2.01	2.42	1.36	0.84
SFC @ 86 F	0.00	1.38	1.67	0.98	0.39
SFC @ 92 F	0.00	0.49	0.78	0.58	0.1
SFC @ 95 F	0.00	0.3	0.56	0.05	0.04
SFC @ 104 F	0.00	0	0	0	0
16:0 sn-2	2.46	19.35	20	12.29	2.67
Total Saturated Fatty Acids (FAs)	27.02	26.96	27.63	27.62	28.33

[71] FIG. 3 shows flow rate change over first half-life of TLIM enzyme for a target Mettler dropping point (“MDP”) of 82-83 degrees Fahrenheit with certain reaction conditions according to aspects of the present invention. In the half-life test, TLIM enzyme was loaded into a heat-jacketed column, and basestock cottonseed oil was passed through the enzyme bed to facilitate continuous enzymatic intraesterification, and the intraesterified cottonseed oil product was then analysed. The MDP and flow rate through the column was measured daily. In the example shown in FIG. 3, the column set-up comprised a TLIM load of 24 grams, a reaction temperature of 70° C, feedstock consisting of RBD cottonseed oil, and the following reaction targets: flow rate began at 60grams/hour, 82-83° F MDP, and 17-19% sn-2 monopalmitin. The following observations were made: (i) the 1st half-life = rate of reaction slowed by 50%, 30grams/hour, to achieve the MDP target; (ii) the 2nd and 3rd half-life was determined by the time taken to reduce flow by 75% and 87%. In an embodiment, a column in a production plant may be run to the 3rd half-life effectively to utilize the enzyme.

[72] The half-life test indicates the (i) effect of oil type and quality on enzymatic reaction efficiency; (ii) how long the enzyme will work to meet reaction targets; and (iii) the amount of oil reacted per gram of enzyme.

[73] Table 5 shows the run conditions for producing intraesterified cottonseed oil in accordance with aspects of the invention. The samples produced were identified as 5995-105, 5995-110, 5995-116, and 5995-67. The run conditions for producing intraesterified cottonseed oil sample 5995-67 was substantially similar to the run conditions for producing intraesterified cottonseed oil sample 5995-105.

Table 5

Blend	CSO Large Bench Regular Flow	CSO Large Bench Fast Flow	CSO Large Bench Double –Fast Flow
ID	EIE DEOD CSO, Large Bench Final Combined Large Bench Deod 15 gal. Homog of Collect 1, 2, 3 5995-105	EIE DEOD CSO, Large Bench Collect 1, Fast Flow (4.5 gal. produced) 5995-110	EIE DEOD CSO, Large Bench Collect 1, Double Fast Flow 5995-116
MI/Min (Optos 1HM pump)	14.55 ml/min	14.55 ml/min	14.55 ml/min
Enzyme	TLIM	TLIM	TLIM
Column Temperature	70° F	70° F	70° F
Actual Grams/Hour	727.41	727.82	730.66
Actual MI/Hour	799.35	799.80	802.92
Enzyme Bulk Density (g/ml)	0.40	0.40	0.40
Enzyme Void Space (g/ml)	0.60	0.60	0.60
Amount Enzyme (g)	267.16	133.58	66.79
Oil Density (ml/g)	0.91	0.91	0.91
Retention Time (minutes)	30.08	15.03	7.49

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[74] Table 6 shows the impact of cotton stearin addition post intraesterification to EIE deodorized CSO made in accordance with the present invention under regular flow (residence time). As shown in Table 6, the addition of stearin provides increased SFC % at 50° F to EIE deodorized CSO as compared to an EIE cottonseed oil devoid of stearin. For example, an EIE deodorized CSO made in accordance the present invention having 10.72% SFC % at 50°F without stearin was increased by (i) 16.5% to 12.84% SFC at 50° F by adding 3% CSO stearin, (ii) 23.4% to 13.99% SFC at 50° F by adding 5% CSO stearin, and (iii)

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29.7% to 15.25% SFC at 50° F by adding 7% CSO stearin. In another example, an EIE deodorized CSO made in accordance the present invention having 11.97% SFC at 50°F without stearin was increased by (i) 18% to 14.59% SFC % at 50° F by adding 3% CSO stearin, (ii) 24.9% to 15.94% SFC at 50° F by adding 5% CSO stearin, and (iii) 29.54% to 16.99% SFC at 50° F by adding 7% CSO stearin. Those skilled in the art having the benefit of the present disclosure will recognize that the present invention allows for production of an EIE cottonseed oil having a specific predetermined target SFC% at 50° F by addition of stearin. The ability to fine tune production of an EIE cottonseed oil having a specific predetermined target SFC% at 50° F is useful to meet the needs of manufacturers of margarines and spreads.

Table 6

Analysis	Regular Flows							
	16.72 SFC@50F Start				11.97 SFC@50F Start			
	NO ADD	3% CSO Stearin	5% CSO Stearin	7% CSO Stearin	NO ADD	3% CSO Stearin	5% CSO Stearin	7% CSO Stearin
	EIE Deod CSO Large Bench 3rd Trial Collection 1 Homog 5-gal 5995-67	97% EIE Cottonseed Oil Base Oil 5995-67 Typical Flow (16.72 SFC@50F); 3% Cottonseed Oil Stearin	95% EIE Cottonseed Oil Base Oil 5995-67 Typical Flow (16.72 SFC@50F); 5% Cottonseed Oil Stearin	93% EIE Cottonseed Oil Base Oil 5995-67 Typical Flow (16.72 SFC@50F); 7% Cottonseed Oil Stearin	EIE DEOD CSO Large Bench 5995-105	97% EIE Cottonseed Oil Base Oil 5995-105 Typical Flow (11.97 SFC@50F); 3% Cottonseed Oil Stearin	95% EIE Cottonseed Oil Base Oil 5995-105 Typical Flow (11.97 SFC@50F); 5% Cottonseed Oil Stearin	93% EIE Cottonseed Oil Base Oil 5995-105 Typical Flow (11.97 SFC@50F); 7% Cottonseed Oil Stearin
MDP (°F)	82.40	82.22	82.94	82.40	81.86	82.94	82.94	84.56
SFC @ 50° F	16.72	12.54	13.59	15.25	11.97	14.59	15.94	16.99
SFC @ 68° F	3.49	3.98	4.20	4.17	4.19	4.31	4.77	5.06
SFC @ 70° F	3.07	3.41	3.56	3.97	3.78	3.95	4.95	4.21
SFC @ 77° F	2.30	2.56	2.69	2.66	2.60	2.71	2.83	2.75
SFC @ 80° F	2.01	2.30	2.19	2.17	2.42	2.28	2.42	2.19
SFC @ 88° F	1.39	1.46	1.29	1.56	1.67	1.79	1.74	1.66
SFC @ 92° F	0.49	0.59	0.71	0.86	0.78	0.69	1.00	0.93
SFC @ 95° F	0.30	0.25	0.44	0.17	0.56	0.49	0.66	0.39
SFC @ 104° F	0.00	0.05	0.00	0.14	0.06	0.00	0.10	0.00
Q16.0 sm-2	19.35				26			

[75] Table 7 shows the impact of cotton stearin addition post intraesterification for fast flow, i.e., ½ or 50% regular enzyme amount. As shown in Table 7, the addition of stearin provides increased MDP temperatures and increased SFC % at 50° F under fast flow conditions as compared to an EIE cottonseed oil devoid of stearin under fast flow conditions.

- 5 The ability to fine tune production of an EIE cottonseed oil having a specific predetermined target MDP temperature and SFC% at 50° F is useful to meet the needs of manufacturers of margarines and spreads.

Table 7

Fast Flow (1/2 regular enzyme amount)				
10.45 SFC @ 50° F Start				
	NO ADD	3% CSO Stearin	5% CSO Stearin	7% CSO Stearin
Analytical	EIE DEOD CSO Large Bench Collect 1 Fast Flow 5995-110	97% EIE Cottonseed Oil Base Oil 5995-110 Fast Flow (10.45 SFC@50F): 3% Cottonseed Oil Stearin	95% EIE Cottonseed Oil Base Oil 5995- 110 Fast Flow (10.45 SFC@50F): 5% Cottonseed Oil Stearin	93% EIE Cottonseed Oil Base Oil 5995-110 Fast Flow (10.45 SFC@50F): 7% Cottonseed Oil Stearin
MDP (°F)	77.54	78.80	80.41	78.98
SFC @ 50°F	10.45	10.73	11.14	11.44
SFC @ 68°F	3.00	3.25	3.37	3.28
SFC @ 70°F	2.71	3.01	2.76	3.06
SFC @ 77°F	1.78	1.98	1.90	2.00
SFC @ 80°F	1.36	1.52	1.51	1.77
SFC @ 86°F	0.98	1.00	1.07	0.97
SFC @ 92°F	0.58	0.47	0.48	0.45
SFC @ 95°F	0.05	0.14	0.00	0.03
SFC @ 104°F	0.00	0.00	0.00	0.00
C 16:0 sn-2	12.29			

[76] Table 8 shows the impact of cotton stearin addition post intraesterification for fast flow, i.e., 1/4 or 25% regular enzyme amount. As shown in Table 8, the addition of stearin provides increased SFC % at 50° F under double fast flow conditions as compared to an EIE cottonseed oil devoid of stearin under double fast flow conditions. As noted above, the ability to fine tune production of an EIE cottonseed oil having a specific predetermined target MDP temperature and SFC% at 50° F is useful to meet the needs of manufacturers of margarines and spreads.

Table 8

Double Fast Flow (1/4 regular enzyme amount)				
4.39 SFC @ 50° Start				
	NO ADD	20% CSO Stearin	25% CSO Stearin	30% CSO Stearin
Analytical	EIE DEOD CSO Large Bench Collect 1 Double-Fast Flow 5995-116	80% EIE Cottonseed Oil Base Oil 5995- 116 Double-Fast Flow (4.39 SFC@50F): 20% Cottonseed Oil Stearin	75% EIE Cottonseed Oil Base Oil 5995- 116 Double-Fast Flow (4.39 SFC@50F): 25% Cottonseed Oil Stearin	70% EIE Cottonseed Oil Base Oil 5995-116 Double-Fast Flow (4.39 SFC@50F): 30% Cottonseed Oil Stearin
MDP (°F)	73.22	77.18	78.08	77.9
SFC @ 50°F	4.39	9.34	11.59	14.34
SFC @ 68°F	1.62	2.25	2.63	2.72
SFC @ 70°F	1.55	2.01	2.22	2.55
SFC @ 77°F	1.00	1.45	1.73	1.55
SFC @ 80°F	0.84	1.08	1.30	1.39
SFC @ 86°F	0.39	0.60	0.59	0.76
SFC @ 92°F	0.10	0.00	0.00	0.00
SFC @ 95°F	0.04	0.00	0.00	0.00
SFC @ 104°F	0.00	0.00	0.00	0.00
C 16:0 sn-2	2.67			

[77] The following examples discuss the production of fat spreads in accordance with aspects of the invention. More specifically, 60% fat spreads using EIE Cottonseed Oil were processed to different degrees of reaction. The various fat spreads were analysed to
5 determine the degree of reaction effects have on the crystallization and functionality of EIE cottonseed oil as a base oil in a spreads application. Two spread formulations that have cotton stearin added at different concentrations were produced and analysed to determine the effect of cotton stearin on the physical properties of the base oil.

[78] 60% fat spreads were produced on an Armfield votation unit, then stored at
10 refrigerated temperatures. Characterization testing was performed on the spreads over a set period of time.

Samples Produced:

- [79] Spread #1 (5995-105 Regular Flow)
[80] Spread #2 (5995-67 Regular Flow)
15 [81] Spread #3 (5995-110 Fast Flow)
[82] Spread #4 (5995-110 Fast Flow + 5% Cotton Stearin)
[83] Spread #5 (5995-116 2x Fast Flow + 25% Cotton Stearin)
[84] Spread #6 Control (75% SBO/25% 74-325-0)
[85] Batch size = 4.5 kg for each spread

Sample Collection:

- [86] Spreads were processed on an Armfield votation unit.
[87] Spreads were collected in 16-ounce cup containers, filled 2/3 full of each spread.
These samples were used for the characterization work on the spreads for the duration of the study. In addition, each spread was placed in 4-ounce containers to be used for sensory
25 evaluation over the duration of the study.

Duration of Study:

- [88] 0 week, 1 week, 2 weeks, 4 weeks, and 8 weeks were the time points for testing the spreads after initial production. Protocol for testing spread samples: static texture data was collected on spread right out of fridge, then the spread sample was left out for 1 hour then put
30 back into fridge to recrystallize, and texture data was collected on cycled spread the next day.

Analysis:

[89] Spread base: 50g of spread base (oil blend, distilled monoglycerides, lecithin) was pulled before adding aqueous phase to create an emulsion. On each spread base run the following: SFC, MDP, FAC, ROC.

5 [90] Spreads: Texture Analyzer method using TA-55 5mm Puncture Probe, Sensory Evaluation, Microscopy.

[91] Table 9 shows the formulation for spreads #1 through #6, wherein spreads #1 through #5 were EIE CSO produced in accordance with aspects of the present invention, and spread #6 was a control.

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Table 9

Spread #1 (5995-105 regular flow)	%	4.5 kg Batch	
Oil Phase (60%)			
EIE Cottonseed Oil (lot 5995-105 Regular Flow)	59.30	2.6685 kg	
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		
Spread #2 (5995-67 regular flow)	%	4.5 kg Batch	
Oil Phase (60%)			
EIE Cottonseed Oil (lot 5995-67 Regular Flow)	59.30	2.6685 kg	
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		
Spread #3 (5995-110 fast flow)	%	4.5 kg Batch	
Oil Phase (60%)			
EIE Cottonseed Oil (lot 5995-110 Fast Flow)	59.30	2.6685 kg	
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		
Spread #4 (5995-110 fast flow + 5% Cotton Stearin)	%	4.5 kg Batch	
Oil Phase (60%)			
EIE Cottonseed Oil (lot 5995-110 Fast Flow)	56.335	2.535075 kg	
Cotton Stearin (lot)	2.965	0.133425 kg	= 133.425 g
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		

Spread #5 (5995-116 2x fast flow + 25% Cotton Stearin)	%	4.5 kg Batch	
Oil Phase (60%)			
EIE Cottonseed Oil (lot 5995-116 2x Fast Flow)	44.475	2.001375 kg	
Cotton Stearin (lot)	14.825	0.667125 kg	= 667.125 g
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		
Spread #6 Control (75% SBO/25% 74-325-0)	%	4.5 kg Batch	
Oil Phase (60%)			
Soybean Oil	44.475	2.001375 kg	
IE Palm Stearin/Palm Kernel Oil	14.825	0.667125 kg	= 667.125 g
Distilled Monoglyceride	0.50	0.0225 kg	= 22.5 g
Soy Lecithin	0.20	0.009 kg	= 9 g
	60.00		
Water Phase (40%)			
Water	39.00	1.755 kg	
Salt	1.00	0.045 kg	= 45 g
	40.00		

[92] Table 10 shows an analysis for spread oil phase sample spreads #1 through #6, identified in Table 9. As shown in Table 10, spreads #1 through #5, produced using intraesterified cottonseed oil in accordance with aspects of the present invention, have SFC % at 50° F that is close to spread #6 produced using 75% soybean oil (SBO)/25% interesterified palm stearin/palm kernel oil 74-325-0). For example, spread #1 had SFC % at 50° F of 12.00%, which is close to spread #6, which had SFC % at 50° F of 14.60%. The regular flow rate (referred to as typical flow in Table 10) used for spreads #1 and #2 was the same regular flow rate used for spread #6.

Table 10

Analysis	Spread Oil Phase Samples (contain base oil blend, distilled monoglyceride, and soy lecithin)					
	Spread #1 EIE CSO Typical Flow Lot# 11671	Spread #2 EIE CSO Typical Flow (Different Batch) Lot# 11672	Spread #3 EIE CSO Fast Flow Lot# 11673	Spread #4 EIE CSO Fast Flow + 5% Cotton Stearine Lot# 11674	Spread #5 EIE CSO Double Fast Flow + 25% Cotton Stearine Lot# 11675	Spread #6 Control 75% SBO / 25% EIE Palm/PK Lot# 11676
SFC @ 50° F	12.00	11.05	10.45	11.00	11.98	14.60
SFC @ 70° F	3.49	3.79	3.11	3.27	3.15	6.75
SFC @ 80° F	3.19	2.63	2.20	2.01	2.02	4.47
SFC @ 92° F	1.09	0.85	0.36	0.42	0.07	2.15
SFC @ 104°F	0.25	0.21	0.02	0.13	0	0
Mettler Drop Point (°C)	28.3	28.4	26.8	26.9	26.4	30.6
Mettler Drop Point (°F)	82.94	83.12	80.24	80.42	79.52	87.08
Butyric C4:0	0.00	0.00	0.00	0.00	0.00	0.00
Caproic C6:0	0.00	0.00	0.00	0.00	0.00	0.02
Caprylic C8:0	0.00	0.01	0.01	0.01	0.01	0.38
Capric C10:0	0.00	0.00	0.00	0.00	0.00	0.35
Lauric C12:0	0.02	0.01	0.01	0.01	0.01	4.78
Myristic C14:0	0.89	0.81	0.89	0.87	0.82	1.90
Palmitic C16:0	23.97	23.35	24.07	24.75	28.80	17.80
Palmitoleic C16:1	0.59	0.54	0.59	0.58	0.53	0.07
Stearic C18:0	3.06	3.16	3.03	3.03	2.98	4.70
Total C18:1 trans FA	0.08	0.06	0.05	0.06	0.04	0.05
Total C18:1 cis FA	15.55	16.21	15.52	15.45	15.32	21.48
Total C18:2 trans FA	0.25	0.31	0.28	0.27	0.23	0.11
Total C18 2 cis FA	53.85	53.68	53.79	53.27	49.57	42.01
Total C18:3 trans FA	0.02	0.01	0.01	0.02	0.00	0.13
Total C18:3cis FA	0.27	0.28	0.24	0.19	0.21	5.49
Arachidic C20:0	0.28	0.29	0.27	0.26	0.26	0.30
Behenic C22:0	0.12	0.14	0.13	0.11	0.13	0.24
Lignoceric C24:0	0.09	0.07	0.11	0.08	0.07	0.05
Total trans FA	0.35	0.38	0.33	0.35	0.28	0.28
Total Saturated FAs	28.39	27.80	28.51	29.10	33.05	30.38

[93] The following procedure was used for texture analysis of food spread samples.

Texture attributes of spread samples were measured by using Texture Analyzer TA.HDPlus

5 equipped with a 5mm cylindrical puncture probe (TA-55 5 mm). A test consisted of using a “Return to Start” test measured in compression to penetrate into a tub of product at a test speed of 2 mm/sec, to a depth of 10 mm. Three replicates were tested for each sample and the probe was wiped clean with a lint-free towel between each replicate. The “initial sample” represents measurements done on a product tub pulled from refrigerator (38° F) and tested
10 within 2 minutes of withdrawal.

[94] FIG. 4 depicts a texture analysis model graph and parameter interpretation for a food spread. The following describes the model graph and parameter interpretation.

[95] Firmness/ Hardness (Force, g) – Maximum resistance to the penetration (height of peak 2 in FIG. 4). The higher the maximum resistance, *i.e.*, height of peak 2, the higher is
15 the firmness of the spread.

[96] Spreadability/ Adhesiveness (Force-time, g.s) – This characteristic is indicated primarily by the profile of the negative curves (sharp & narrow or shallow & extended) and the minima of the adhesion peak shown in FIG. 4. During probe withdrawal, if the sample

exhibited more adhesion tendency (sample+probe) than cohesion (sample+sample), then the profile of negative curve will be shallow and extended and the negative curve area will be small. The larger the negative curve area, the higher the brittle nature and the lower of its spreadability of the spread. For comparable negative curve areas, a “sharp and narrow”
 5 profile represents brittle nature and a “shallow and extended” profile represents relative ease of spreadability of the spread.

[97] Consistency (Force-time, g.s) – This characteristic is indicated primarily by the profile of the resistance peak (smooth or jagged) and the area of the resistance peak. A smooth graph indicates product uniformity at different depths of container. Jaggedness
 10 indicates varying resistance to the descending probe stemming most likely from development of shear planes to relieve the buildup of internal stress.

[98] FIG. 5 is a graph showing spread firmness as a function of ageing for spreads comprising intraesterified cottonseed triglyceride product in accordance with aspects of the present invention as compared to a control spread comprising 75% soybean oil and 25%
 15 interesterified palm stearin/palm kernel oil, as characterized by peak force (g) versus time (weeks). As shown in FIG. 5, spreads #1 through #5 comprising intraesterified cottonseed triglyceride product had greater spread firmness from week zero (0) through week eight (8) than the control spread #6. Spread #1 exhibited the most consistency of spread firmness over the eight (8) week trial than the other spreads, followed closely by spread #2. At week
 20 zero (0), spreads #1 and #2 had spread firmness of about 110 peak force (g) and 125 peak force (g), respectively, whereas the control spread had spread firmness of about 40 peak force (g). At week eight (8), spreads #1 and #2 had spread firmness of about 110 peak force (g), whereas the control spread had spread firmness of about 70 peak force (g). Spreads #3, #4 and #5 demonstrate the impact of adding cotton stearin to the intraesterified cottonseed oil to
 25 increase spread firmness. Spreads #1 and #2 showed very consistent firmness throughout the aging study indicating that lot to lot intraesterified cottonseed oil demonstrates similar crystallization tendencies or characteristics. The consistency over the 8-week storage study of spread #1, spread #2 and spread #6 indicate that intraesterified cottonseed oil demonstrates similar crystallization behaviour to the of the interesterified palm / palm kernel basestock
 30 commonly used in margarine and spread manufacturing.

[99] FIG. 6 is a graph showing spread spreadability as a function of ageing for spreads comprising intraesterified cottonseed triglyceride product in accordance with aspects of the present invention as compared to a control spread comprising 75% soybean oil and 25%
 interesterified palm stearin/palm kernel oil, as characterized by peak force (g) versus time

(weeks). Spread #1 exhibited the most consistency of spread firmness over the eight (8) week trial than the other spreads, followed closely by spread #2. At week zero (0), spreads #1 and #2 had spread spreadability of about -50 peak force (g) and -55 peak force (g), respectively, whereas the control spread had spread spreadability -20 peak force (g). At week eight (8), spreads #1 and #2 had spread spreadability of about -45 peak force (g) and -40 peak force (g), respectively, whereas the control spread #6 had spread spreadability of about -25 peak force (g). Spreads #1 and #2 demonstrate similar spreading characteristics to spread #6 over the course of the 8-week storage study. This data indicates that the post crystallization tendencies of the intraesterified cottonseed oil are similar to that common interesterified palm / palm kernel oil based spread commonly used in the margarine and spreads industry.

[100] FIG. 7 is a graph showing rate of crystallization at 50° F as characterized as SFC (% solids at 50° F) as a function of time for spreads comprising intraesterified cottonseed triglyceride product (spreads #1 through #5) in accordance with aspects of the present invention as compared to a control spread comprising 75% soybean oil and 25% interesterified palm stearin/palm kernel oil (spread #6). Spreads #1 through #5 showed similar crystallization rates, demonstrating that intraesterified cottonseed oil with or without cotton stearin adjustment equilibrates similar to that of the spread #6 basestock after 24 hours. This data indicates that within 24 hours intraesterified cottonseed oil demonstrates similar crystallization characteristics to a spread made with interesterified palm / palm kernel oil commonly used in margarine and spread production.

[101] FIG. 8 is a graph showing rate of crystallization at 50° F as characterized as SFC (% solids at 50° F) as a function of time for intraesterified cottonseed triglyceride product in accordance with aspects of the present invention. FIG. 8 shows the impact of flow rates on rate of crystallization. The intraesterified cottonseed triglyceride products identified in FIG. 8, i.e., EIE CSO LG Bench Deod Typical Flow 5995-109 (typical flow is also referred to herein as regular flow), EIE CSO LG Bench Deod Fast Flow 5995-110, and EIE CSO LG Bench Deod Db1 (Double) Fast Flow 5995-116, were deodorized and did not contain cotton stearin. As shown in FIG. 8, rate of crystallization characteristics will vary depending on the degree of intraesterification that takes place a result of the flow rate and residence time. The data shown in FIG. 8 further indicates that intraesterified cottonseed oil in accordance with aspects of the present invention can be successfully used as feedstock or basestock in margarine or spread production.

[102] The addition of saturated fat, e.g., in the form of cotton stearin, was found to increase ability to fine tune characteristics of the product comprising intraesterified cottonseed oil.

[103] The addition of cotton stearin improved spread resiliency to thaw-freeze cycle.

[104] In accordance with aspects of the invention, cotton stearin may be added before
5 intraesterification or after intraesterification of the basestock.

[105] Different crops of natural cottonseed oil can have a different amount of saturated components in the triglyceride, e.g., different total palmitic acid and total saturates. For example, over a three (3) year period, different crops of natural cottonseed oil have been observed that have saturated components varying from about 27.6 to about 24.9% by weight.

10 In an aspect of the present invention, the addition of cotton stearin prior to enzymatic intraesterification may be used to adjust saturate content to ensure greater consistency in feedstock.

[106] In accordance with aspects of the present invention, enzyme half-life can be improved by re-bleaching and/or re-deodorizing RBD oil to improve oil quality prior to
15 intraesterification of the RBD oil.

[107] Those skilled in the art having the benefit of the present disclosure will recognize that the above features disclosed herein provides processes and compositions that do not have the disadvantages of conventional processes and compositions.

[108] Those having skill in the art, with the knowledge gained from the present disclosure,
20 will recognize that various changes can be made to the disclosed processes in attaining these and other advantages, without departing from the scope of the present disclosure. As such, it should be understood that the features of the disclosure are susceptible to modifications and/or substitutions. The specific embodiments illustrated and described herein are for illustrative purposes only, and not limiting of the invention as set forth in the appended
25 claims.

WHAT IS CLAIMED IS:

1. A composition comprising a 1,3-selective enzymatic intraesterified oil product having a greater USU content than the oil from which it was derived.
2. The composition of claim 1, wherein U is primarily linoleic acid and the S is palmitic acid.
3. The composition of claim 1, wherein the 1,3-selective enzymatic intraesterified oil product is 1,3-selective enzymatic intraesterified cottonseed oil product.
4. A vegetable oil spread comprising water, an emulsifying agent, and at least 5% by weight of the 1,3-selective enzymatic intraesterified oil product of claim 1.
5. An intraesterified non-tropical triglyceride product having a solid fat content at 50°F that is greater than the basestock from which it was derived.
6. The intraesterified non-tropical triglyceride product of claim 5, wherein the intraesterified non-tropical triglyceride product has a solid fat content at 50°F that is at least ten (10) times greater than the basestock from which it was derived.
7. The intraesterified non-tropical triglyceride product of claim 5 having a solid fat content of at least 4% at 50°F.
8. The intraesterified triglyceride product of claim 5 having a solid fat content of at least 7% at 50°F.
9. The intraesterified triglyceride product of claim 5 having a solid fat content of at least 9% at 50°F.
10. The intraesterified triglyceride product of claim 5 having a solid fat content of at least 9.5% at 50°F.
11. The intraesterified triglyceride product of claim 5 wherein the main unsaturated fatty acid is linoleic acid.
12. The intraesterified triglyceride product of claim 5 wherein the intraesterified triglyceride product is an intraesterified cottonseed oil.
13. The intraesterified cottonseed triglyceride product of claim 5 wherein the product has re-esterified fatty acids derived solely from cottonseed oil.
14. An intraesterified cottonseed triglyceride product having a melting temperature of at least 75°F.
15. The intraesterified cottonseed triglyceride product of claim 14 wherein the product has re-esterified fatty acids derived solely from cottonseed oil.
16. A vegetable oil spread comprising water, an emulsifying agent, and at least 5% of the intraesterified cottonseed triglyceride product of claim 14.

17. An intraesterified cottonseed triglyceride product having at least 25% more palmitic acid esterified to the second carbon of the glycerol than the basestock from which it was derived.
18. The intraesterified cottonseed triglyceride product of claim 17 with at least 12%
5 palmitic acid esterified to the second carbon of the glycerol.
19. A vegetable oil spread comprising water, an emulsifying agent, and at least 5% of the intraesterified cottonseed triglyceride product of claim 17.
20. A method for increasing the USU content in an oil, comprising performing 1,3-selective enzymatic intraesterification on a regular non-tropical \ starting oil wherein
10 SUS content is greater than the USU content in the starting oil prior to performing 1,3-selective enzymatic intraesterification.
21. The method of claim 20, wherein the regular non-tropical starting oil is a single oil extracted from an oil source and not blended with other oils.
22. The method of claim 20, wherein the unsaturated U in USU is primarily linoleic acid.
- 15 23. The method of claim 20, wherein the saturated S in USU is primarily palmitic acid.
24. The method of claim 14, wherein the oil is cottonseed oil.
25. The method of claim 20, wherein the method is a continuous method.
26. The method of claim 20, wherein the 1,3-selective enzymatic intraesterification is performed by using lipase enzymes.
- 20 27. The method of claim 20, wherein the lipase enzyme comprises a lipase from *Thermomyces lanuginosis* (TLIM).
28. A composition comprising a 1,3-selective intraesterified oil obtained by performing the method of claim 20, wherein the 1,3-selective intraesterified oil is enriched in USU as compared to the oil it was produced from.
- 25 29. The composition of claim 28, wherein the 1,3-selective intraesterified oil is 1,3-selective intraesterified cottonseed oil.

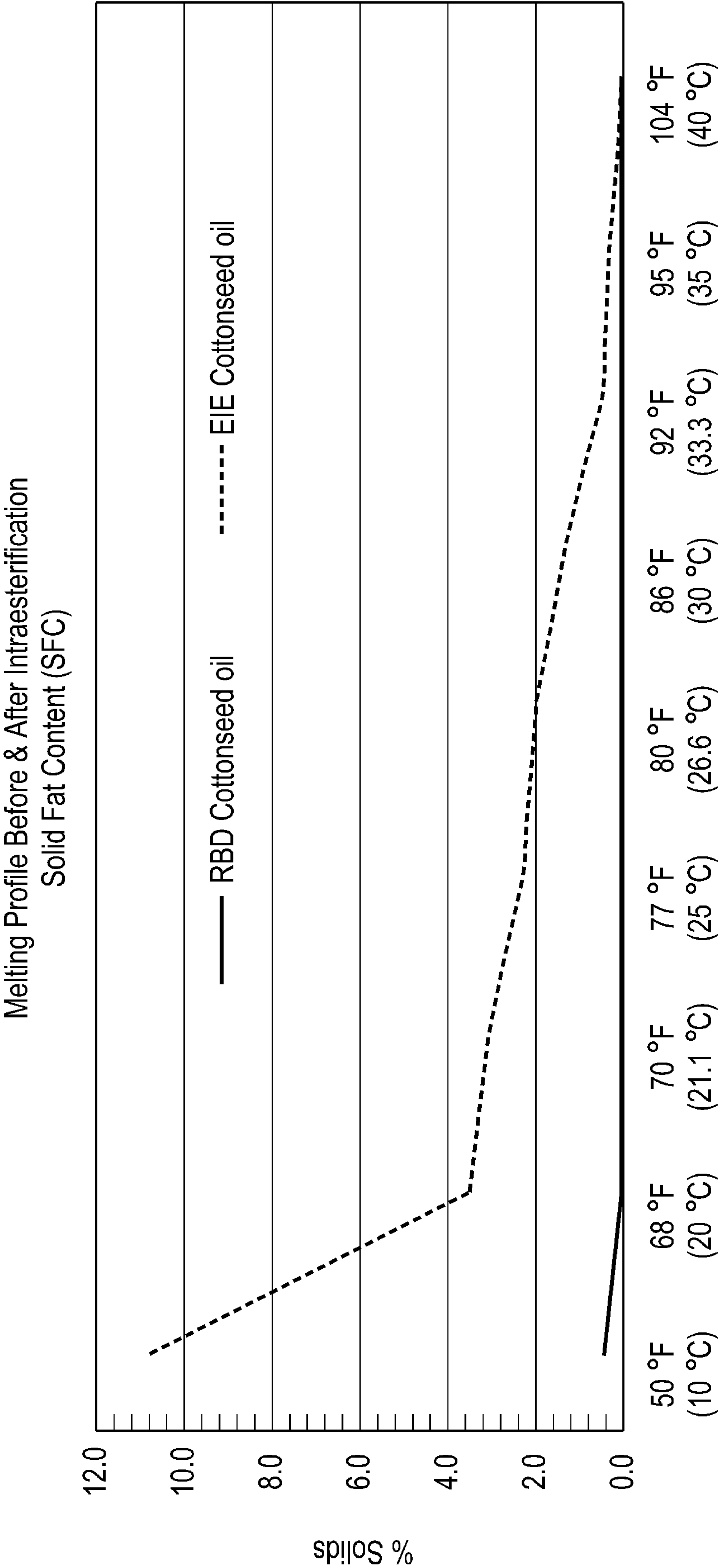


FIG. 1

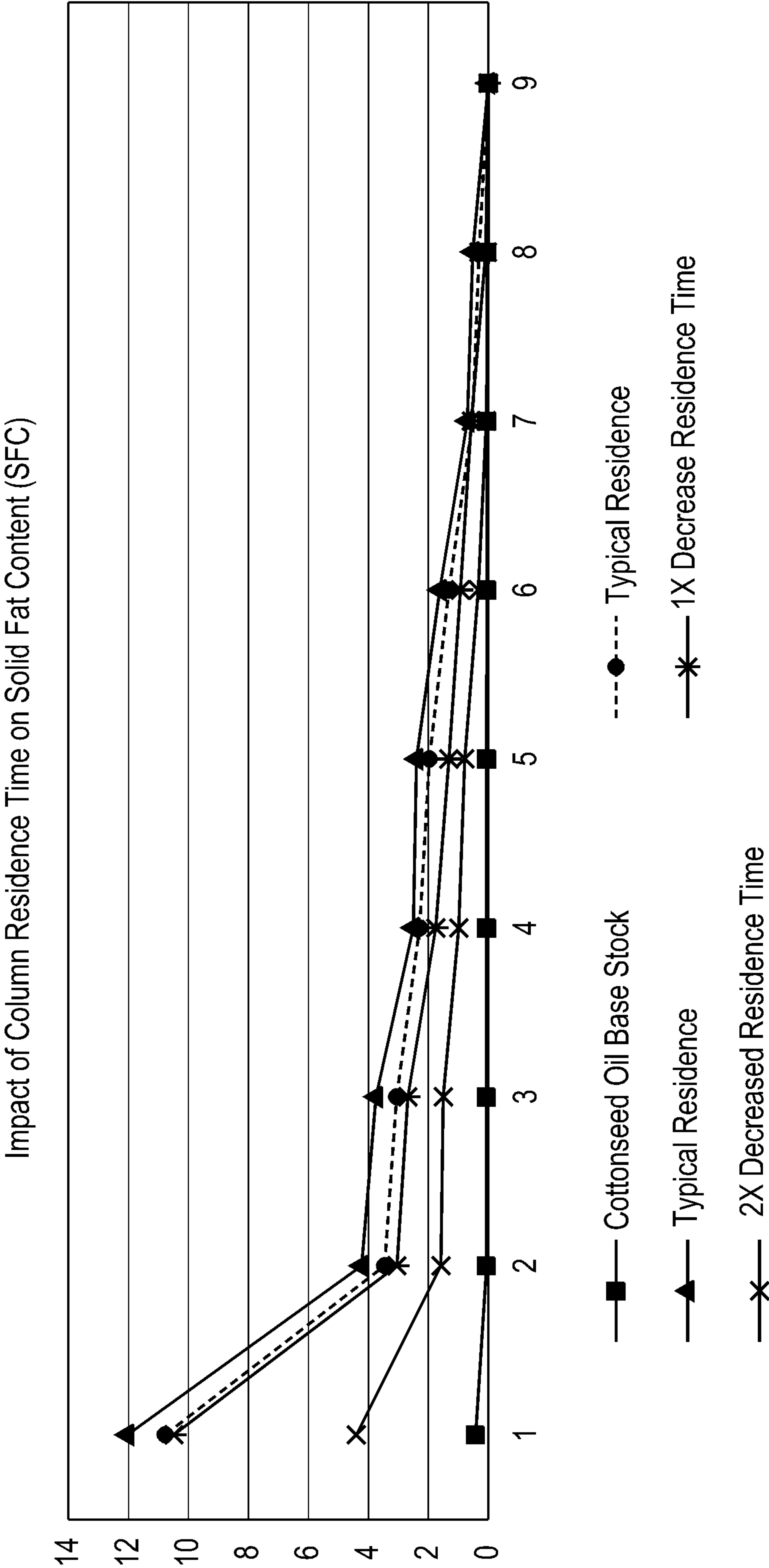


FIG. 2

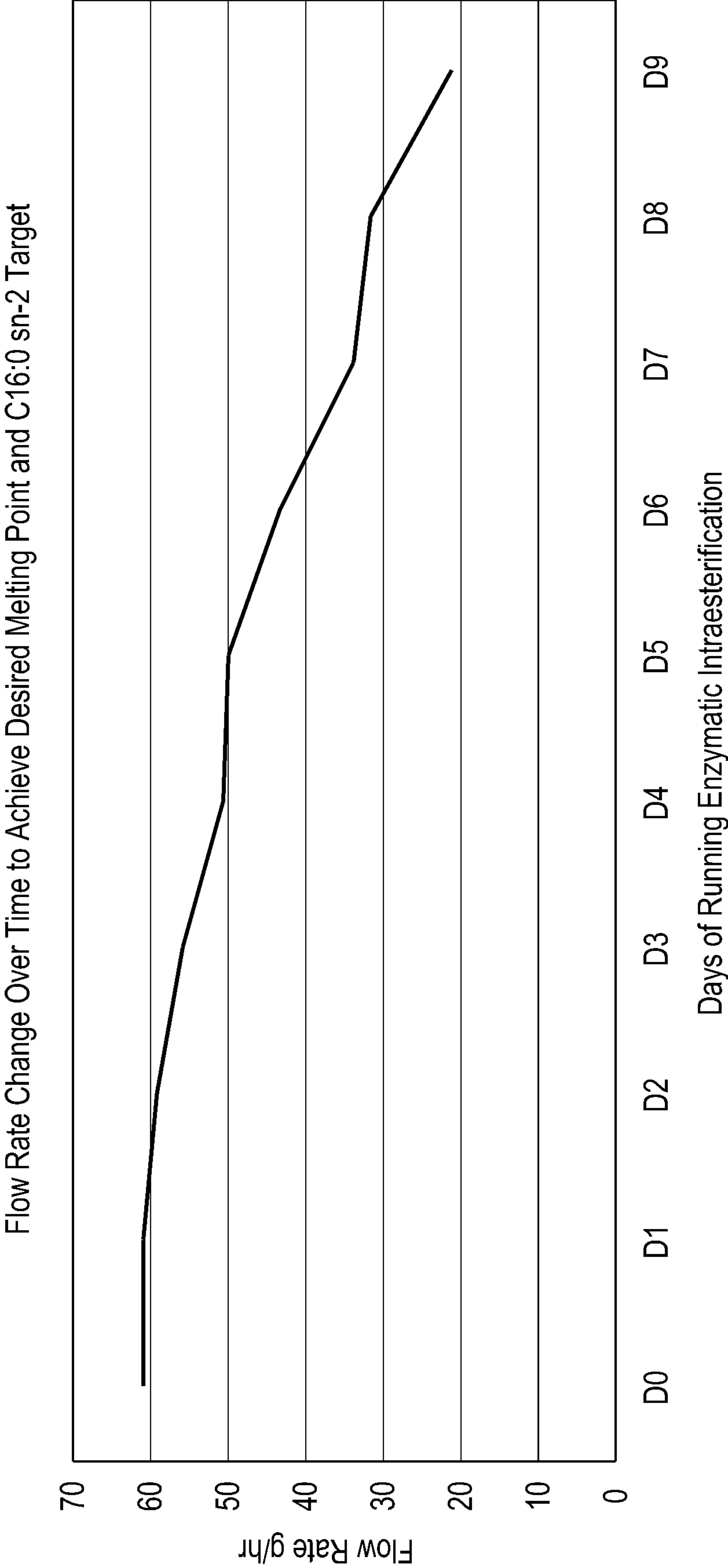


FIG. 3

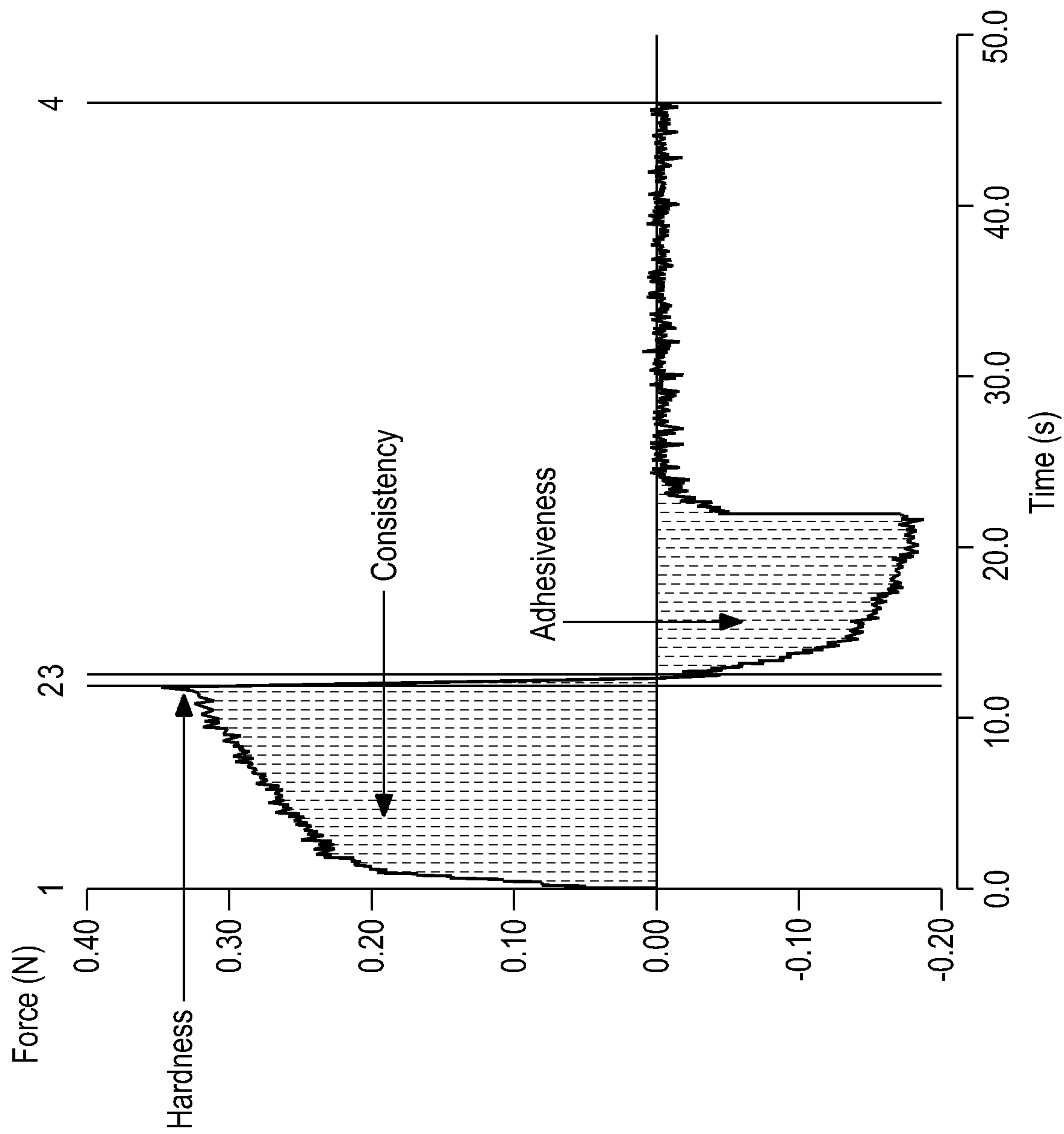


FIG. 4

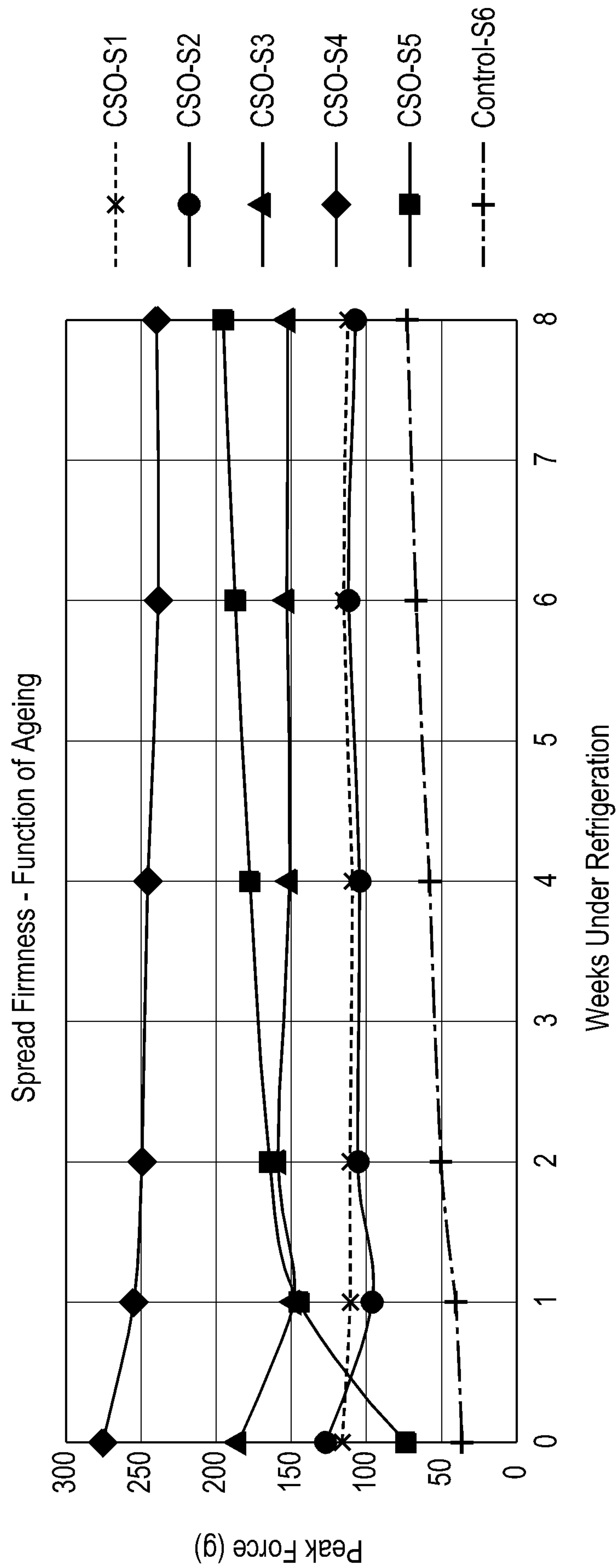


FIG. 5

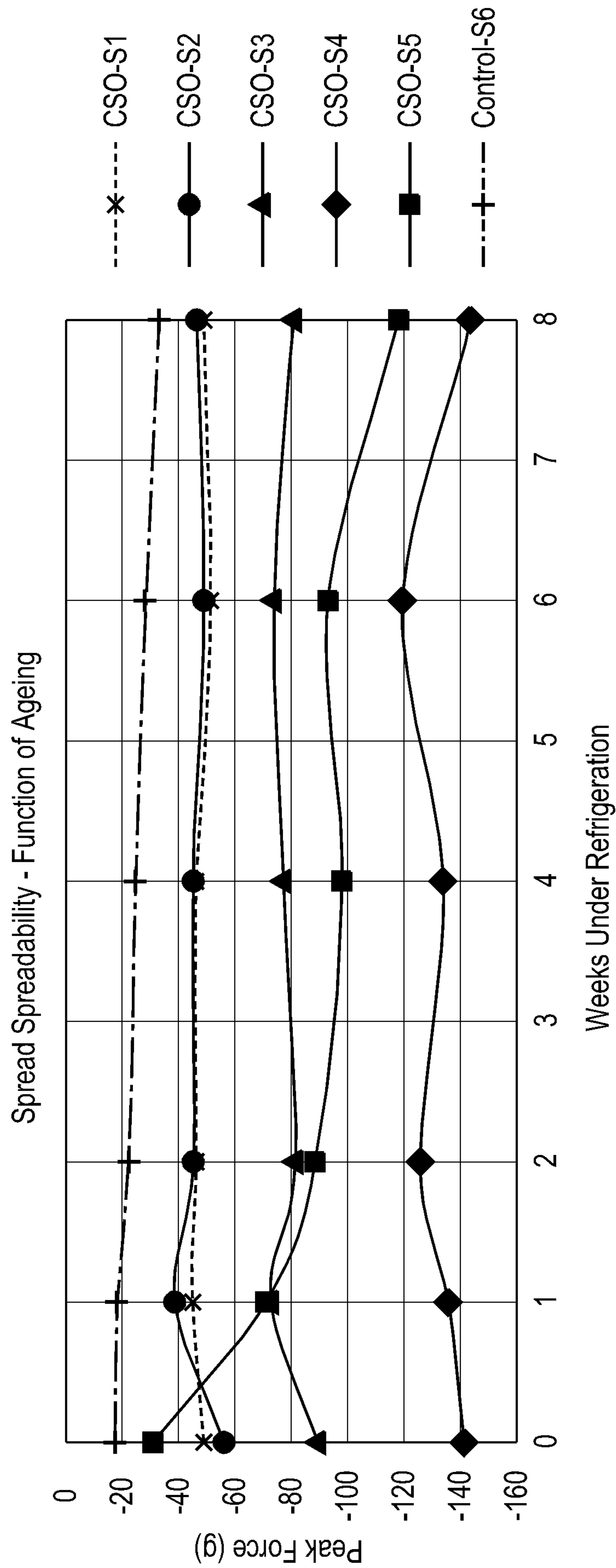


FIG. 6

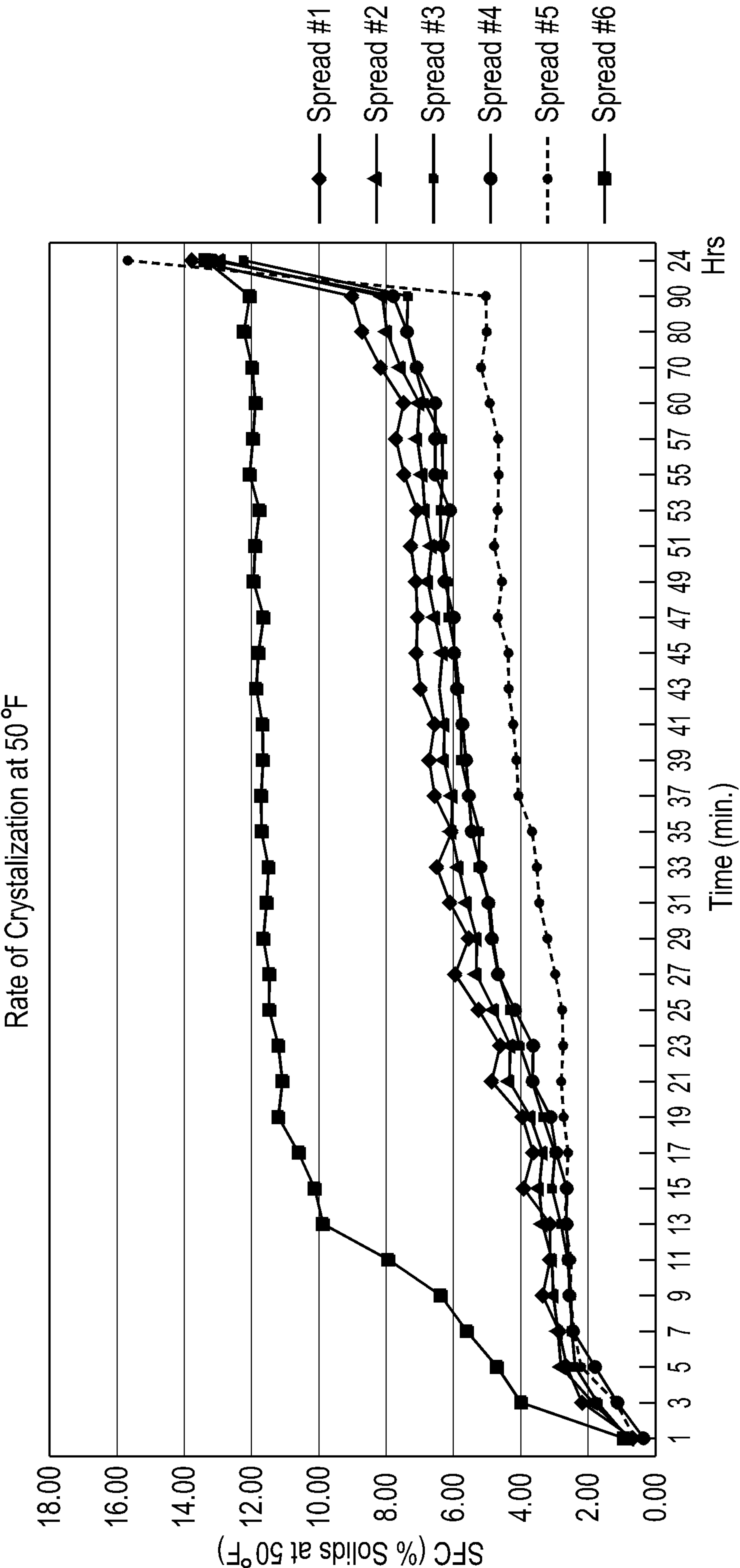


FIG. 7

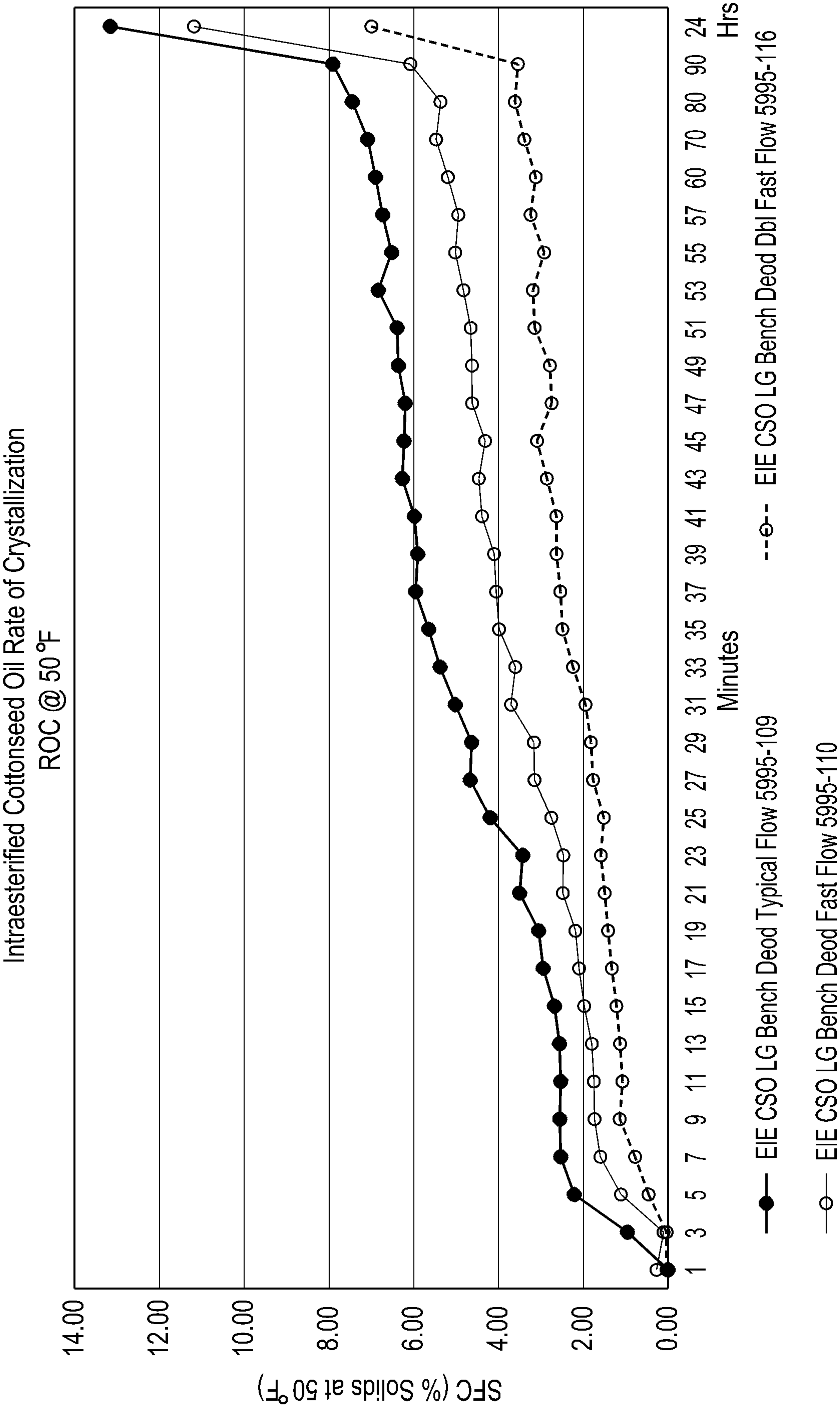


FIG. 8

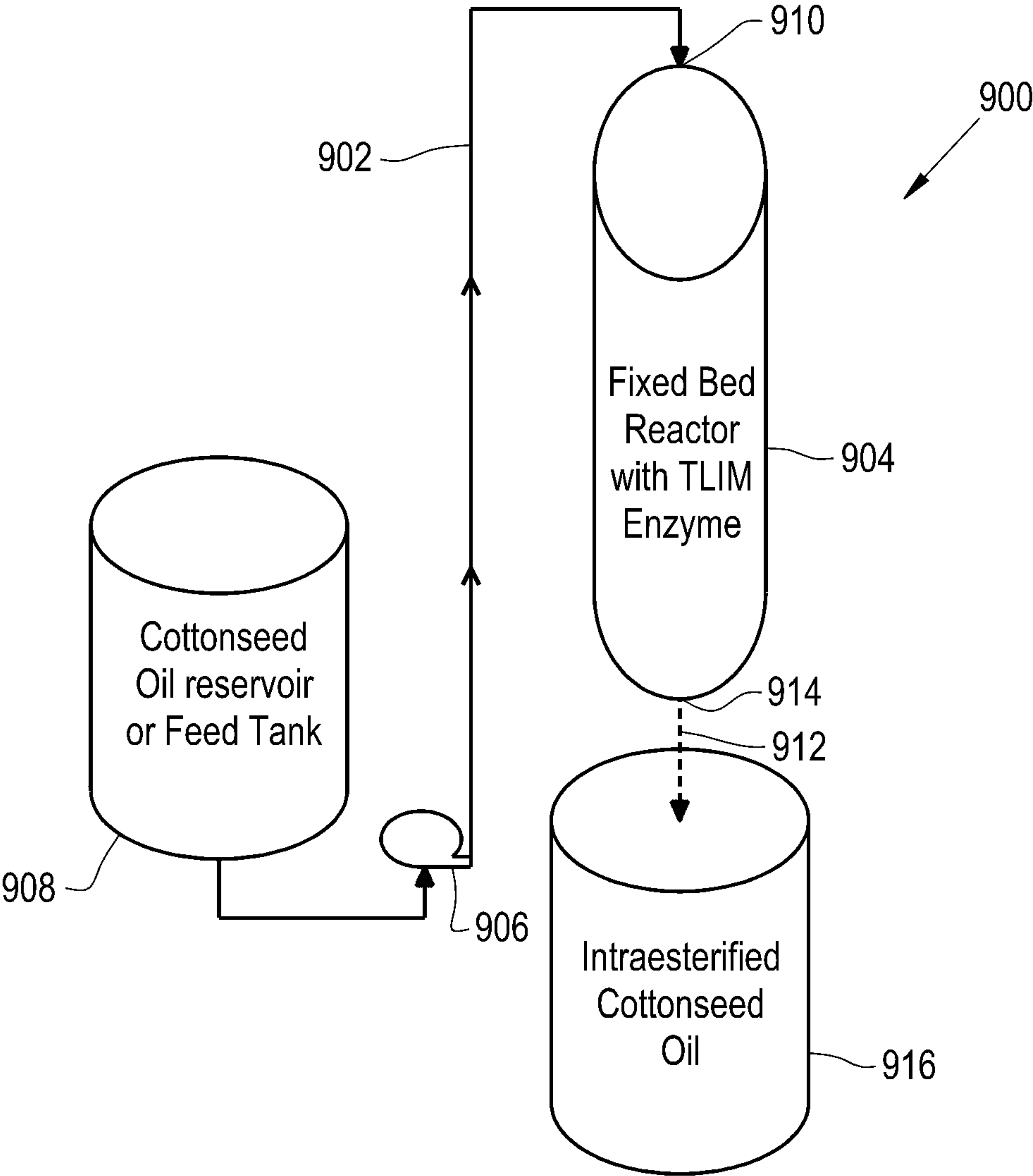


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/12972

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C11C 1/04; C12P 7/64; A61K 35/60 (2021.01)

CPC - C11C 1/045; C12P 7/6409; C12P 7/6418

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2014/016250 A1 (Advanta International BV) 30 January 2014 (30.01.2014), Figure 4, pg 10, ln 32-33, pg 11, ln 1-2, pg 6, ln 8-11, abstract, Figure 5, pg 4, ln 16-21, pg 3, ln 1-3, pg 5, ln 29, pg 8, ln 22-24, pg 3, ln 26-31, pg 10, ln 19-21, Table 1, pg 14, ln 16-18, pg 14, ln 7	1-3, 5-15, 17-18, 20-29 ----- 4, 16, 19
Y	US 9,795,151 B2 (General Mills, Inc.) 24 October 2017 (24.10.2017), entire document especially abstract, col 12, ln 31-32, col 12, ln 34-35	4, 16, 19
A	US 2018/0030485 A1 (Fuji Oil Holdings Inc.) 1 February 2018 (01.02.2018), entire document	1-29
A	US 2011/0045555 A1 (Kralovec et al.) 24 February 2011 (24.02.2011), entire document	1-29
A	US 2015/0166932 A1 (Advanta International BV) 18 June 2015 (10.06.2015), entire document	1-29
A	US 4,567,056 A (Schmidt) 28 January 1986 (28.01.1986), entire document	1-29

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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"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

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Date of the actual completion of the international search

10 March 2021

Date of mailing of the international search report

APR 01 2021

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