



(51) International Patent Classification:

C11C 3/14 (2006.01) C07C 51/353 (2006.01)
C07C 51/347 (2006.01)

(21) International Application Number:

PCT/US2014/015139

(22) International Filing Date:

6 February 2014 (06.02.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/762,678 8 February 2013 (08.02.2013) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: CONJUGATED LINOLEIC ACID RICH VEGETABLE OIL PRODUCTION

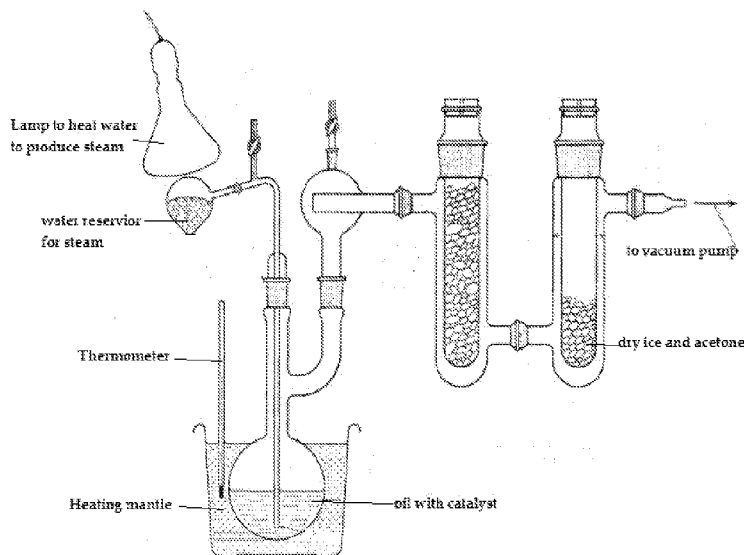


FIG. 1

(57) Abstract: The invention is generally directed to conjugated linoleic acid (CLA)-rich vegetable oil produced from linoleic rich oils by heterogeneous catalysis. The process includes heterogeneous catalytic vacuum distillation under high temperature conditions to isomerize linoleic acid in triacylglyceride vegetable oils to CLA to produce CLA-rich oils. After processing, the catalyst may be removed to obtain high quality, CLA-rich oils. The CLA-rich oils may then serve as a potent and bioactive nutraceutical and can be incorporated into various food products, such as a CLA-rich dressing, shortening, margarine, chocolate or chips.

CONJUGATED LINOLEIC ACID RICH VEGETABLE OIL PRODUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 61/762,678, filed February 8, 2013, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

[0003] This invention relates generally to conjugated linoleic acid (CLA)-rich vegetable oil production from linoleic rich oils by heterogeneous catalysis, and in particular to a process for producing CLA-rich oil by isomerizing linoleic acid in triacylglyceride vegetable oils to CLA by low pressure/high temperature catalysis.

2. Description of the Related Art.

[0004] CLA is a group of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. CLA has anti-carcinogenic, anti-atherogenic, anti-diabetic and anti-obesity properties, along with the ability to increase lean body mass and to protect against immune induced body wasting disease, chronic inflammatory disease, cancer and to provide other positive health effects.

[0005] CLA is found naturally in dairy and beef products at levels of approximately 0.3-0.8% (w/w) of the fat as bovine rumen fermentation products. The current human intake of CLA

is, however, approximately ten (10) times less than the 3g/day minimum value recommended as being necessary to produce desirable physiological health effects. Obtaining the estimated optimum dietary CLA levels from natural beef and dairy sources would increase the total fat and saturated fat intake and increase the negative health risks associated with dietary animal fats. Therefore, a concentrated source of dietary CLA that is low in saturated fat and cholesterol is desirable.

[0006] Soy oil is the most commonly used vegetable oil in United States, and it contains about 50% linoleic acid. Other vegetable oils high in linoleic acid include sunflower (57%), corn (55%), cottonseed (50%) and peanut (50%). CLA fatty acid has been produced historically by fermentation and enzyme technology. CLA in vegetable oil has also been produced by converting linoleic acid to CLA using iodine by homogeneous photo-catalysis. A drawback of this process is the removal of iodine in order for the resulting CLA-rich oil to be suitable for human consumption.

[0007] It is therefore desirable to provide CLA-rich vegetable oil produced from linoleic rich oils using heterogeneous catalysis.

[0008] It is further desirable to provide a process for producing CLA-rich oil by isomerization of linoleic acid in triacylglyceride vegetable oils to CLA using low pressure/high temperature catalysis.

[0009] It is still further desirable to provide a process for producing a 20% CLA-rich oil that requires only post-processing catalyst removal.

[0010] It is yet further desirable to provide a process for rapidly producing CLA-rich oils from linoleic rich oils using heterogeneous catalysis that may utilize a continuous fixed bed reactor and/or a continuous stirred tank reactor for a cost effective and energy efficient process.

[0011] It is yet further desirable to provide a process for producing CLA-rich oils from linoleic rich oils using heterogeneous catalysis that is an environmentally-friendly process in contrast to alkali isomerization.

[0012] It is still further desirable to provide a process for producing CLA-rich oils from linoleic rich oils using heterogeneous catalysis that does not require any solvent or any chemical other than catalyst.

[0013] It is yet further desirable to provide a process for producing CLA-rich oils from linoleic rich oils using heterogeneous catalysis in the absence of iodine and with a metal catalyst that can be easily removed and reused.

[0014] It is yet further desirable to provide a process for producing CLA-rich oils from linoleic rich oils using heterogeneous catalysis that utilizes continuous steam injection to remove any rancidity volatiles in linoleic acid-rich oil.

BRIEF SUMMARY OF THE INVENTION

[0015] In general, the invention relates to a process for producing conjugated linoleic acid-rich oil. The process includes catalyzing an oil-catalyst mixture of linoleic acid-rich oil and a catalytic amount of a transition metal having a vacant 3d-orbital or a vacant 4d-orbital to produce the conjugated linoleic acid-rich oil. The process can also include the step of extracting the metal from the conjugated linoleic acid-rich oil, such as via filtration or centrifugation. The linoleic acid-rich oil can be a triacylglyceride vegetable oil, such as soy, sunflower, corn, cottonseed or peanut oil.

[0016] The catalysis of the process can further include catalyzing the oil-catalyst mixture in low pressure conditions and high temperature conditions to produce the conjugated linoleic acid-rich oil. The oil-catalyst mixture can be catalyzed for up to approximately 240 minutes to produce the conjugated linoleic acid-rich oil. Further, the catalysis can be processed between approximately 1 mm Hg and approximately 2 mm Hg at temperature conditions between approximately 165 °C and approximately 282 °C. Furthermore, the transition metal can be ruthenium, rhodium, molybdenum, palladium, gold, copper, iron, manganese, silver or nickel having a concentration between 0.08% to approximately 8%, such as a ruthenium catalyst or a nickel catalyst.

[0017] In addition, the invention relates to a conjugated linoleic acid-rich oil produced by the process described herein. Moreover, the invention relates to an enriched food product prepared using the conjugated linoleic acid-rich oil from the process described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 is a schematic diagram of an example of a deodorization process in an aspect of the CLA-rich vegetable oil production from linoleic rich oils by heterogeneous catalysis disclosed herein;

[0019] Figure 2 is a contour plot predicted by a statistical model showing various processing conditions obtained for optimum CLA yields by isomerization of soy oil linoleic acid to CLA by ruthenium catalysis, where: the contour lines are a function of time and temperature along which the function has a constant value of CLA yields (18, 18.5, 19, 19.5 and 20%); the unshaded area shows the combination of conditions that can be used to obtain CLA yields of $\geq 18.5\%$; and the data was obtained from a high temperature, long processing time study using a central composite rotatable experimental design to optimize heterogeneous ruthenium catalysis of 250 mL soy oil linoleic acid to CLA with 0.64% ruthenium catalyst over a range of temperatures (197-282 °C) and times (108-192 min);

[0020] Figure 3 is a graphically illustration of the effect of ruthenium on the CLA yield at 270 °C for 3 hours with 4% formic acid in oil and steam of 85 % formic acid;

[0021] Figure 4 is a comparative graphical illustration of force versus compressive extension for soy oil margarine, CLA-rich soy oil margarine and commercial margarine, where five repetitions were made for each sample and the standard deviation was within 5 % of mean load values in all cases;

[0022] Figure 5 is a graphical illustration of complex modulus (G^*) measured as a function of oscillatory stress for control soy oil margarine, CLA-rich soy oil margarine and commercial margarine, where the test was performed in triplicates and error bars represent standard deviation;

[0023] Figure 6 is a graphical plot of change in phase angle with applied oscillatory stress measured for control soy oil margarine, CLA-rich soy oil margarine and commercial margarine, where the test was performed in triplicates and error bars represent standard deviation;

[0024] Figure 7 is DSC thermograms showing the melting profiles of control soy oil margarine, CLA-rich soy oil margarine and commercial margarine;

[0025] Figure 8 is a graphical illustration of the solid fat content measured as a function of temperature for control soy oil margarine, CLA-rich soy oil margarine and commercial margarine measured in triplicate and error bars represent standard deviation;

[0026] Figure 9 are control soy oil margarine microscopic images observed under transmitted normal (9a1) and polarized (9a2) light CLA-rich soy oil, margarine microscopic images observed under transmitted normal (9b1) and polarized (9b2) light, and commercial margarine microscopic images observed in transmitted normal (c1) and polarized (c2) light; and

[0027] Figure 10 is a graphical illustration of puncture force tests (N) of CLA-rich soil oil shortening samples.

[0028] Other advantages and features of the invention will be apparent from the following description and from the claims.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The processes and compositions discussed herein are merely illustrative of specific manners in which to make and use this invention and are not to be interpreted as limiting in scope.

[0030] While the processes and compositions have been described with a certain degree of particularity, it is to be noted that many variations and modifications may be made in the details of the sequence, components, concentrations and the arrangement of the processes and compositions without departing from the scope of this disclosure. It is understood that the invention is not limited to the embodiments set forth herein for purposes of exemplification.

[0031] The invention is generally directed to a heterogeneous catalytic vacuum distillation process that utilizes high temperature conditions to isomerize linoleic acid in triacylglyceride vegetable oils to CLA in order to produce CLA-rich oils. After processing, the catalyst may be removed by filtration or centrifugation to obtain high quality, CLA-rich oils. The heterogeneous catalysis utilizes a metal catalyst that can be selected from any suitable transition metal, such as ruthenium, rhodium, silver or nickel. The process is a two-phase system (oil/catalyst) using conditions with low pressure, between approximately 1 to 2 mm of Hg pressure, high temperature, above about 200 °C, and a continuous flow, such as at about 0.4 ml/min steam flow, to produce a 20% CLA-rich oil in less than 2 hours. The CLA-rich oils may then serve as a potent and bioactive nutraceutical. In addition, the CLA-rich oils can be incorporated into various food products, such as a CLA-rich dressing, shortening, margarine, chocolate or chips.

EXAMPLES

[0032] The CLA-rich vegetable oil production from linoleic rich oils by heterogeneous catalysis disclosed herein is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation. Although soy oil was used in the following examples, any linoleic acid-rich oil can be used.

EXAMPLE 1

[0033] Studies were conducted in duplicate to screen nickel, ruthenium, silver and rhodium catalysts for isomerization of linoleic acid to CLA. Rhodium on carbon with 5% loading (Sigma-Aldrich Product number: 206164), ruthenium (Sigma-Aldrich Product number: 206180), nickel on silica/alumina ~65 wt. % loading (Sigma-Aldrich Product number: 208779). Silver powder instead of silver coated on carbon was used because silver is not commercially available coated on carbon or other support.

[0034] Catalyst concentrations and processing conditions are in Table 1 below. Duplicate samples of 250 mL of fully refined oil were processed for 90 minutes at their optimum temperature of each catalyst. Control experiments were also conducted using iodine (0.35%) and tocopherols (0.14%) catalysts dissolved in oil.

Table 1: Commercial metal catalysis screening for CLA-rich oil production relative to production with iodine and mixed tocopherols.

	Table 1 Treatments	Conc. of catalyst (%)	Temp (°C)	Ratio (c:t:t,t)	Total mean CLA(%)	PV	FFA(% as oleic)
●	IODINE AND MT						
1	Iodine	0.35	255	1.2	27.50±0.12	0.64	0.040
●	MIXED TOCOPHEROLS						
2	Mixed Tocopherols	0.14	255	0.17	1.84±0.42	0.24	0.030
3	Mixed Tocopherols	1.40	255	.006	1.813±0.32	0.32	0.032
●	RUTHENIUM						
1	Ruthenium on carbon(5% load)	0.08	255	0.85	4.14±0.35	0.41	0.042
2	Ruthenium on carbon (5% load)	0.08	165	0.86	4.05±0.23	0.39	0.034
3	Ruthenium on carbon(5% load)+ 0.14% MT	0.08	165	1.51	5.21±0.44	0.34	0.032
4	Ruthenium on carbon (5% load)	0.32	165	3.03	10.52±0.86	0.38	0.041
5	Ruthenium on carbon(5% load)+1400 MT	0.32	165	2.80	9.043±0.62	0.32	0.032
6	Ruthenium on carbon(5%load)	0.48	165	2.73	13.78±0.81	0.55	0.04
7	Ruthenium on carbon(5%load)	0.64	165	2.18	14.972±0.83	0.62	0.042
●	RHODIUM						
1	Rhodium on carbon(5% load)	0.015	200	0.17	2.37±0.30	0.52	0.032
2	Rhodium on carbon(5% load)	0.32	200	1.34	10.83±0.45	0.73	0.05
●	OTHERS						
1	Silver powder 1000 mesh(10 mic)	1	200	0.007	1.46±0.25	0.38	0.021
2	Nickel 400 mesh or 37 mic	1	200	0.01	1.10±0.22	0.43	0.023
3	Nickel (55% loading on carbon)	1	200	0.24	1.831±0.32	0.50	0.035

[0035] Heterogeneous catalyzed oils were then filtered and oil fatty acid composition determined by GC fatty acid methyl ester analysis in duplicate. Oil quality was determined by measuring peroxide value and free fatty acid value in duplicate.

[0036] As the data in Table 1 illustrates, ruthenium and rhodium produced higher but statistically similar CLA levels at a concentration of 0.32% than other metal catalysts. Since rhodium is 10 times more expensive than ruthenium, ruthenium was selected as the most viable metal for CLA production by heterogeneous catalytic deodorization process. Ruthenium was further optimized as an economically viable conjugation catalyst, and preliminary screening showed that increasing the ruthenium catalyst concentration increases CLA yields. Around 15% CLA was produced at a catalyst level of 0.64% at 165 °C over 90 min.

[0037] Transition metals contain free and vacant d-orbitals, which can interact with pi-bonds of linoleic acid, and which are capable of activating a nearby C-H bond leading to bond migration. Such interaction destabilizes the native structure allowing the more stable CLA conjugated system to form and be released from the catalyst.

EXAMPLE 2

[0038] To optimize conditions by a central composite rotatable experimental design using a Ruthenium catalyst (Ru loaded on carbon) was used with 5 levels of 3 variables (catalyst concentration, time, and temperature) (Table 2). The results of Example 1 were used to select the center point for experimental design to determine the effect of variables on CLA yields above and below the center point.

Table 2: Central Composite Rotatable Design of Example 2 for Optimization of CLA Production Over a Broad Range of Temperatures (90-240 °C), Times (40-140 min) and Ru Catalyst Concentrations (0.21-0.75%).

Variables	Levels				
	-1.682	-1	0	1	1.682
Temperature(°C)	90	120	165	210	240
Time (min)	40	60	90	120	140
Catalyst Concentration (%)	0.21	0.32	0.48	0.64	0.75

Sr. no.	Catalyst Conc.(%)	Time(min)	Temperature(°C)	%CLA (rep 1)	%CLA (rep 2)	% CLA mean	SD	C.I.t.t ratio	PV	FFA
1	0.48	140	165	11.09	10.78	10.94	± 0.22	2.562	0.32	0.031
2	0.48	40	165	6.42	6.10	6.26	± 0.23	2.327	0.34	0.040
3	0.48	90	240	14.43	14.02	14.23	± 0.29	1.518	0.34	0.032
4	0.64	120	130	2.34	2.22	2.28	± 0.06	0.54	0.42	0.050
5	0.75	90	165	6.82	6.60	6.71	± 0.15	2.139	0.32	0.053
6	0.32	120	120	2.36	2.26	2.31	± 0.07	0.56	0.48	0.054
7	0.32	120	210	13.23	13.44	13.33	± 0.15	1.98	0.28	0.024
8	0.21	90	165	3.64	4.02	3.83	± 0.26	7.49	0.38	0.028
9	0.64	60	120	0.17	0.15	0.16	± 0.01	(not)	0.40	0.055
10	0.48	90	165	9.84	9.33	9.59	± 0.36	5.38	0.38	0.020
11	0.48	90	90	0.15	0.16	0.16	± 0.01	No t	0.54	0.056
12	0.32	60	210	12.24	11.74	11.99	± 0.36	3.822	0.34	0.043
13	0.48	90	165	10.20	9.58	9.89	± 0.44	5.36	0.32	0.023
14	0.32	60	120	0.17	0.16	0.17	± 0.01	No t	0.49	0.054
15	0.64	60	210	16.89	16.14	16.51	± 0.53	1.944	0.36	0.034
16	0.64	120	210	20.87	19.93	20.41	± 0.65	2.20	0.42	0.043

[0039] The ruthenium catalyst is on a carbon support consisting of a 19 microns particle with total surface area surface area of 900 m² per gram. The ruthenium catalyst has a surface area of 13 m² per gram and moisture content of <5% (Sigma-Aldrich Product number: 206180).

[0040] Two hundred and fifty milliliter samples were processed in duplicate for each set of processing conditions. Oils were then filtered and oil fatty acid composition determined by gas chromatography free fatty acid (FFA) methyl ester analysis in duplicate. Oil quality was determined by measuring the peroxide value (PV) and free fatty acid value in duplicate.

[0041] As the data in Table 2 illustrates, 0.64% ruthenium at 210 °C over 120 minutes produced 20% CLA-rich oil, which were the conditions that produced most CLA. Yields increased with temperature, time and catalyst concentration but time and temperature had the greatest effect on CLA yields.

EXAMPLE 3

[0042] To further optimize the time and temperature conditions for a catalyst concentration fixed at 0.64% (ruthenium on carbon), a central composite rotatable design was utilized with 5 levels of 2 variables (time and temperature) (Table 3). The results of Example 2 were used to select the higher temperature and time settings for the experimental design to determine the effect of variables on CLA yields for time and temperature conditions over the ones used in Example 2. The objective of Example 3 was based on the hypothesis predicted by experimental design of Example 2 that higher temperatures and times would give greater CLA yields at a fixed catalyst concentration (0.64%), as further loading of the catalyst coated on carbon increased the viscosity of oil which would increase process cost and cause filtration problems.

Table 3: Central Composite Rotatable Design for Example 3 for Optimization CLA-Rich Oil Production Over a High Temperature (197-282 °C) Range and Longer Times (108-192 min) than in Example 2, with 0.64% Ru catalyst.

Variables	Levels				
	-1.682	-1	0	1	1.682
Temperature(°C)	197	210	240	270	282
Time (min)	108	120	150	180	192

No.	time(min)	temperature	CLA (rep1)	CLA (rep 2)	mean	SD	Ratio (c,t,t,t)	PV	FFA
1	180	210	17.36	16.54	16.95	0.58	1.68	0.43	0.05
2	108	240	20.90	21.20	21.05	0.21	1.75	0.44	0.04
3	180	270	18.62	19.60	19.11	0.69	1.51	0.65	0.06
4	192	240	17.56	17.10	17.33	0.33	1.08	0.53	0.06
5	150	282	18.48	18.20	18.34	0.20	1.35	0.64	0.05
6	150	240	17.05	17.45	17.25	0.28	0.93	0.49	0.05
7	120	210	19.04	19.60	19.32	0.40	2.51	0.45	0.05
8	150	197	14.90	15.34	15.12	0.31	2.09	0.36	0.04
9	150	240	17.58	17.10	17.34	0.34	0.95	0.45	0.05
10	120	270	17.80	17.46	17.63	0.24	0.99	0.68	0.06

[0043] As the data in Table 3 illustrates, CLA yields at higher temperatures and longer times with 0.64% ruthenium catalyst resulted in less CLA produced relative to Example 2. A maximum of 21.05% CLA can be obtained at 240 °C temperature for 108 min.

[0038] An additional study was conducted to optimize conditions of a nickel catalyst for isomerization of linoleic acid to CLA using a central composite rotatable design similar to the preceding examples. The nickel catalyst was used on a silica/alumina (powder) with total surface area surface area of 190 m² per gram (Sigma Aldrich Product number: 208779). The nickel catalyst concentration was fixed at 8% with the time and temperature processing conditions listed below in Table 4.

Table 4: Central Composite Rotatable Design of Example 4 for Optimization of CLA Production Over a Temperature Range (180-240 °C) and Times (180-240 min) with 8% Ni catalyst.

Time (min)	Temp (°C)	% CLA yield
240	210	1.55 ± 1.06bc
240	180	0.31 ± 0.19d
210	210	0.49 ± 0.06cd
180	210	0.71 ± 0.10cd
180	240	4.70 ± 2.00a
210	240	2.11 ± 0.22b
240	240	1.96 ± 0.16b
210	180	0.31 ± 0.21d
210	210	0.73 ± 0.09cd
180	180	0.28 ± 0.18d

[0039] As the data in Table 4 illustrates, 8% nickel at 240 °C over 180 minutes produced approximately 6.70% CLA-rich oil, which were the conditions that produced most CLA. Yields increased with temperature, time and catalyst concentration but time and temperature had the greatest effect on CLA yields. While the about 6.7% CLA yields from Example 4 using a nickel catalyst were lower than the around 21% CLA yields from Example 3 that utilized a ruthenium catalyst, nickel is less expensive than ruthenium. Therefore nickel is also an economically viable catalyst with a high commercial potential for CLA production by a heterogeneous catalytic deodorization process.

[0044] In addition to the transition metals of ruthenium, rhodium, silver and nickel utilized in the foregoing non-limiting examples, the processes and systems to produce CLA-rich oils disclosed herein can be used with other inexpensive (comparative to nickel) transitional metal catalysts, such as copper, iron, and manganese having vacant 3d-orbitals. Moreover, more expensive transition metals (comparative to ruthenium), such as molybdenum, palladium and gold having vacant 4d-orbitals and larger atoms than nickel, can similarly be used as catalysts in the processes and systems to produce CLA-rich oils disclosed herein.

EXAMPLE 4

[0045] Example 4 optimizes the operating parameters to maximize CLA yields of CLA-rich oil ruthenium heterogeneous catalysis without the use of solvents or hydrogen and also determines fatty acid distribution and CLA isomer composition of CLA-rich oil produced under optimized conditions. Processing conditions were optimized to maximize CLA yields using central composite experimental design studies with variable time, temperature and catalyst concentration. In Example 4, the inventive process produced 21% CLA-rich oil in 108 min at 240 °C by a single heterogeneous catalysis step using a ruthenium (0.64%) under low pressure (1-2 mm Hg) in presence of steam (0.4 mL/min steam flow).

[0046] Refined, bleached, and deodorized (RBD) soy oil was obtained from Riceland Foods (Stuttgart, AR, USA) and used throughout this Example 4. Ruthenium catalyst (5% loading on carbon) was purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, USA). The lab scale deodorization unit was modeled by O'Brien scientific glassblowing (Monticello, IL, USA) in accordance with the design adapted from Riceland Foods (Stuttgart, AR, USA). HPLC solvents hexane and acetonitrile were HPLC grade (VWR International, West Chester, PA, USA). Other chemicals used were reagent grade.

[0047] *Initial Study with Variable Temperatures, Processing Times and Catalyst Concentration.* A central composite rotatable experimental design was employed to optimize conditions to maximize CLA yields. The design had 5 levels of 3 variables: catalyst concentration (0.21, 0.32, 0.48, 0.64 and 0.75%), time (40, 60, 90, 120 and 140 min) and temperature (90, 120, 165, 210 and 240 °C) in duplicate as shown in Table 5 below. Two hundred and fifty (250) mL soy oil samples were processed in duplicate for each set of processing conditions in a random order as described below.

Table 5: Initial study (Example 4) using Central Composite Rotatable experimental design to optimize heterogeneous ruthenium catalysis of 250-mL soy oil linoleic acid to CLA over a range of temperatures (90-240 °C), times (40-140 min) and catalyst concentrations (0.21-0.75%).

Treatment	Catalyst Conc. (%)	Time (min)	Temperature (°C)	Total CLA (%)	PV (meq/kg)	FFA (%)
1	0.48	140	165	10.94 ± 0.22 _{ab}	0.32	0.03
2	0.48	40	165	6.26 ± 0.23 _c	0.34	0.04
3	0.48	90	240	14.23 ± 0.29 _d	0.24	0.03
4	0.64	120	120	2.28 ± 0.08 _c	0.42	0.05
5	0.75	90	165	6.71 ± 0.15 _b	0.32	0.05
6	0.32	120	120	2.31 ± 0.07 _d	0.48	0.05
7	0.32	120	210	13.33 ± 0.15 _f	0.28	0.02
8	0.21	90	165	3.83 ± 0.26 _g	0.38	0.03
9	0.64	60	120	0.16 ± 0.01 _f	0.40	0.05
10	0.48	90	165	9.59 ± 0.36 _g	0.38	0.02
11	0.48	90	90	0.16 ± 0.01 _g	0.54	0.05
12	0.32	60	210	11.99 ± 0.36 _a	0.34	0.04
13	0.48	90	165	9.89 ± 0.44 _{bh}	0.32	0.03
14	0.32	60	120	0.17 ± 0.01 _g	0.49	0.05
15	0.64	60	210	16.51 ± 0.53 _i	0.36	0.03
16	0.64	120	210	20.41 ± 0.65 _j	0.42	0.04

* Mean CLA yields with same letter are not significantly different by Tukey's significance test.

[0048] The lab scale deodorization unit was used to produce CLA-rich oil by heterogeneous catalysis and is shown in Figure 1. The assembly was composed of glass units with standard ground glass spherical joints that could be lubricated with vacuum grease to maintain a vacuum of 1-2 mm Hg. Steam was generated at a rate of 0.4 mL/min by a 250-watt infrared bulb that heated the water in the water reservoir. The 250-mL round bottom flask placed in the heating mantle contained the mixture of oil and catalyst to be processed. A thermometer was inserted through a ground glass joint into the body of the glass to continuously monitor temperature and the condensers were cooled with the dry ice-acetone mixture. All the vapors were retained by the first condenser and the second condenser acted as a safety device in case of

failure of the first condenser. The outlet of the condenser was attached to the vacuum pump to maintain a near vacuum of 1 to 2 mm Hg pressure throughout the assembly.

[0049] After the oil-catalyst mixture was added to the flask, the dry ice and acetone were added to the condensers (*see* Figure 1) and water was placed in the water reservoir flask. The heating mantle and the vacuum pump were then turned on. As the oil temperature reached 60 °C, the infrared heating lamp was turned on and the pressure valve at the top of the flask was closed. The oil-catalyst mixture was processed for the predetermined specified time and temperature. The oil was then allowed to cool to 30-40 °C in the vacuum. It was then separated from the catalyst by centrifugation at 9000 RPM for 15 min and filtered through a 47 mm Ø circle Whatman microfiber filter. Each processed oil sample was then subjected to the following analysis.

[0050] *Free Fatty Acid Analysis.* The percent free fatty acids were determined in duplicate for each sample replicate, as oleic acid.

[0051] *Peroxide Value Analysis.* Peroxide values were determined in duplicate for each sample replicate by the micro-method.

[0052] *TAG Fatty Acid and CLA Isomer Analysis by FAMES GC-FID.* The fatty acid composition of each replicate sample was determined in duplicate by GC FAMES analysis. Methyl esters were prepared by a base-catalyzed methylation method. One-hundred (100) mg samples were weighed into 25-mL centrifuge tubes and 500 µL of 1% heptadecanoic acid methyl ester (17:0, internal standard), 2 mL of toluene and 4 mL of 0.5 M sodium methoxide in methanol were added to each centrifuge tube and the tubes were purged with nitrogen gas. The centrifuge tubes were heated to 50 °C for 10 min and then cooled for 5 min. After the tubes cooled, 200 µL of glacial acetic acid was added to each centrifuge tube to prevent the formation

of sodium hydroxide. Five (5) ml of distilled water was added to each centrifuge tube followed by 5 mL of hexane, and the tubes were vortexed for 2 min. The hexane layers were extracted and dried over anhydrous sodium sulfate in 7-mL glass vials. The extracted layers were then taken from the glass vial and placed in gas chromatograph vials. Methyl esters were analyzed by GC by using an SP 2560 fused silica capillary column (100 m x 0.25 mm i.d. x 0.2 μ m film thickness; Supelco Inc., Bellefonte, PA, USA) with a flame ionization detector (model 3800, Varian, Walton Creek, CA, USA). Two μ L samples, prepared in hexane, were injected by using an autosampler CP8400 (Varian), and gas chromatograms were collected by Galaxie chromatography workstation 1.9.3.2 (Varian). Analysis of variance was used to determine significant differences in total CLA levels between treatments.

[0053] *Variable Temperatures, Processing Times and Catalyst Concentration.* Table 5 above shows the CLA yields obtained by the variables in the central composite rotatable design experiment. The conditions that produced the most CLA (20%) were a concentration of 0.64% ruthenium, a temperature of 210 °C and a processing time of 120 min. Treatments at temperatures ≤ 120 °C produced less than 3% CLA, independent of time and catalyst concentration. There was significant increase in the CLA content by 3 to 7% in treatments with temperatures of 165 °C compared to treatments with temperatures ≤ 120 °C. The oil CLA increased in the treatments with the temperatures ≥ 210 °C. The increase from 120 to 210 °C resulted in an 8 to 17 % increase in CLA, showing that catalytic isomerization increased with temperature, independent of time and concentration. The effect of time in increasing CLA levels became more significant at higher temperatures. The statistical model (Table 5) showed that there were no interaction effects between the variables on the CLA yields, which also predicted increase in CLA yields with temperature, time and catalyst concentration. However, temperature

had the greatest linear effect on CLA yields. This was followed by time and catalyst concentration effect, respectively.

[0054] *TAG Fatty Acid and CLA Isomer Analysis by FAMES GC-FID.* Table 6 below shows the total fatty acid composition produced in CLA-rich oil processed during the initial study with variable temperatures, processing times and catalyst concentration. There are no significant differences amongst treatments within palmitic, stearic and oleic acid content. However, there was a significant reduction in linoleic and linolenic acid, which were isomerized during processing. These differences were greatest at higher temperature treatments with most CLA production. *Trans,trans* CLA composition also significantly increased with increasing temperature. This occurs because the higher temperatures favors formation of the thermodynamically stable *trans,trans* CLA isomers.

Table 6: Total fatty acid composition of the processed oils obtained in an initial study (Example 4) using Central Composite Rotatable experimental design to optimize heterogeneous ruthenium catalysis of 250-mL soy oil linoleic acid to CLA over a range of temperatures (90-240 °C), times (40-140 min) and catalyst concentrations (0.21-0.75%).

Treatment	Catalyst Conc. (%)	Time (min)	Temperature (°C)	Fatty Acid composition (%) of processed oils*						
				C 16:0	C 18:0	C 18:1	C 18:2 LA	C 18:3	c11/c6 CLA	c11 CLA
1	0.48	140	165	12.51 _a	4.75 _a	23.61 _a	45.19 _{ef}	3.98 _{cdef}	7.87 _{bcd}	3.07 _{cd}
2	0.48	40	165	11.69 _a	4.43 _a	22.71 _a	50.51 _{bcd}	5.39 _{abc}	4.37 _{de}	1.89 _{def}
3	0.48	90	240	14.35 _a	5.38 _a	26.12 _a	37.79 _{gh}	2.13 _{ef}	8.56 _b	5.67 _{ab}
4	0.64	120	120	11.30 _a	4.15 _a	22.02 _a	53.74 _{abc}	6.50 _{ab}	0.8 _{def}	1.48 _{efg}
5	0.75	90	165	11.96 _a	4.49 _a	23.10 _a	48.90 _{de}	4.83 _{abcd}	4.57 _{ce}	2.14 _{de}
6	0.32	120	120	11.09 _a	4.20 _a	21.79 _a	53.89 _{abc}	6.71 _a	0.76 _{ef}	1.37 _{efg}
7	0.32	120	210	13.57 _a	5.09 _a	24.84 _a	40.24 _g	2.81 _{def}	8.86 _b	4.47 _{bcd}
8	0.21	90	165	12.04 _a	4.25 _a	23.29 _a	51.30 _{abcd}	5.52 _{abc}	3.38 _{ef}	0.45 _f
9	0.64	60	120	11.25 _a	4.04 _a	22.79 _a	54.48 _{ab}	6.22 _{ab}	0.16 _f	0 _h
10	0.48	90	165	12.29 _a	4.39 _a	23.96 _a	49.76 _{cd}	4.65 _{abcde}	8.09 _{bcd}	1.5 _{efg}
11	0.48	90	90	11.35 _a	4.07 _a	22.87 _a	55.15 _a	6.39 _{ab}	0.16 _f	0 _h
12	0.32	60	210	13.04 _a	4.74 _a	24.91 _a	42.27 _{fg}	3.05 _{cdef}	9.5 _b	2.49 _{de}
13	0.48	90	165	12.69 _a	4.55 _a	23.52 _a	50.21 _{bcd}	4.44 _{abcde}	8.33 _{bc}	1.56 _{def}
14	0.32	60	120	11.38 _a	4.11 _a	22.86 _a	55.11 _a	6.36 _a	0.16 _f	0 _h
15	0.64	60	210	14.84 _a	5.29 _a	26.84 _a	34.75 _h	1.74 _f	10.7 _{ab}	5.49 _{ab}
16	0.64	120	210	14.49 _a	5.29 _a	26.50 _a	31.58 _i	1.43 _f	13.7 _a	6.23 _a

* Means of respective fatty acid compositions in a column with same letter are not significantly different by Tukey's significance test

[0055] *Free Fatty Acid*. The free fatty acid levels of all treatments were less than 0.06% (Table 5). These low levels were maintained due to volatilization of any FFA formed during the process.

[0056] *Peroxide Value*. The peroxide values of all the treatments were below 1 meq/kg (Table 5), which is within acceptable limits of good quality edible oil. Although oxidation can

occur rapidly when oils containing metal catalyst are exposed to air at high temperatures, peroxide values were minimized by maintaining high vacuum in the processing unit and allowing oil to cool under vacuum in the assembly before filtration. The process was conducted under conditions similar to deodorization processing so volatile oxidation products were readily removed to maintain low oxidation levels.

[0057] As noted above, the effect of higher temperatures and longer processing times, which were not studied in Example 4, needed to be subsequently investigated in an attempt to further increase CLA yields. The Example 4 study with variable temperatures, processing times and catalyst concentration showed that higher temperatures had the most significant effect on maximizing CLA yields, followed by longer processing times. Hence, a high temperature, long processing time study (Example 5 and Table 6) was then conducted to determine the effects of longer times and higher temperatures on CLA yields. However, the catalyst concentration was kept constant at 0.64% as higher concentrations of the catalyst loaded on carbon increased the viscosity of oil which would increase process cost and cause filtration problems.

EXAMPLE 5

[0058] The central composite rotatable experimental design of Example 4 produced a statistical model that predicted that higher temperatures, longer times and greater catalyst amounts would produce greater CLA yields (Table 5 above). The catalyst concentration, however, could not be increased over 0.64% because of an unmanageable increase in oil viscosity. Therefore, a subsequent optimization study (Example 5) was performed at higher temperatures and longer processing times but with a fixed catalyst concentration.

[0059] *High Temperature, Long Processing Time Study.* A central composite rotatable design with 5 levels of 2 variables, time (108, 120, 150, 180 and 192 min) and temperature (197,

210, 240, 270 and 282 °C), was used. Two hundred and fifty (250) mL soy oil samples were processed in duplicate, in random order, for each set of processing conditions, as shown in Table 7.

Table 7: High temperature, long processing time study (Example 5) using a Central Composite Rotatable experimental design to optimize heterogeneous ruthenium catalysis of 250mL soy oil linoleic acid to CLA with 0.64% ruthenium catalyst over a range of temperatures (197-282 °C) and times (108-192 min).

Treatment	Time (min)	Temperature (°C)	CLA yield* (%)	PV (meq/kg)	FFA (%)
1	180	210	16.95 ± 0.58 _a	0.43	0.05
2	108	240	21.05 ± 0.21 _b	0.44	0.04
3	180	270	19.11 ± 0.69 _{cd}	0.65	0.06
4	192	240	17.33 ± 0.33 _a	0.53	0.06
5	150	282	18.34 ± 0.20 _{acd}	0.64	0.05
6	150	240	17.25 ± 0.28 _a	0.49	0.05
7	120	210	19.32 ± 0.40 _d	0.45	0.05
8	150	197	15.12 ± 0.31 _c	0.36	0.04
9	150	240	17.34 ± 0.34 _a	0.45	0.05
10	120	270	17.63 ± 0.24 _{ac}	0.68	0.06

* Mean CLA yields with same letter are not significantly different by Tukey's significance test.

[0060] Each duplicate sample was analyzed in duplicate for free fatty acids, peroxide value and TAG fatty acid composition by FAMES by GC-FID. Analysis of variance was used to determine significant differences in total CLA levels between treatments. Contour profile analysis generated by the central composite design model was used to predict the range of time and temperatures to obtain the optimal CLA levels.

[0061] *CLA Isomers Determination by Silver-ion HPLC.* The positional *trans,trans* CLA and the other CLA isomers were quantified by silver-ion HPLC chromatography, as GC-FID FAMES fatty acid analysis does not distinguish between positional *trans,trans* CLA isomers.

[0062] Silver-ion chromatographic separation, quantification and identification was performed in duplicates for each replicate sample. Two ChromSpher 5 Lipids analytical silver-

impregnated columns each 4.6 mm i.d. x 250 mm stainless steel; 5 μ m particle size (Chrompack, Bridgewater, NJ, USA) in series were used. The column temperature was kept constant at 23 °C by using a temperature control module (Waters Corporation, Milford, MA, USA). About forty μ g of irradiated sample FAMES in 20 μ l hexane was injected using the Waters 717 plus autosampler and a Waters model 600 system, equipped with a quaternary pump (Waters Delta 600), pumping at rate of 1 mL/min. A simple solvent system consisting of 0.1% acetonitrile in hexane was used. The column effluent was connected to a photodiode array (PDA) detector (Waters Model 2996), measuring absorbance at 233 nm. The data output from PDA was integrated by Waters EmpowerTM 2 Software.

[0063] *Statistical Analysis.* Statistical software JMP 10 (SAS Institute, Cary, NC) was used throughout Example 5 to randomize the treatment runs, analyze the results of the central composite design, generate a contour profile of the data and to conduct a three factor regression analysis and analysis of variance ($P=0.05$). The means of the treatments were compared with Tukey's significance test for $P=0.05$.

[0064] *High Temperature, Long Processing Time Results.* Table 6 shows that there is an increase in CLA yields produced relative to the initial study of Example 4. Treatment 2, with levels of 240 °C and 108 min, produced the highest CLA yield of 21.05%, which was significantly higher than other treatments. The statistical model produced from central composite rotatable design showed that, at higher temperatures and longer times there are significant interaction effects between the time and temperature. There is a significant linear and quadratic time effect and a significant linear temperature effect ($P=0.05$).

[0065] As illustrated in Figure 2, the reduced statistical model generated a contour profile to determine the range of times and temperatures that produced the optimal CLA levels. Figure

2 shows the contour lines as a function of time and temperature along which the function has a constant value of CLA yields. Figure 2 shows the contour lines at CLA yields of 18, 18.5, 19, 19.5 and 20 %. The unshaded area shows the combinations of time and temperature necessary to obtain $\geq 18.5\%$ CLA yields.

[0066] *Free Fatty Acid*. The free fatty acid levels of all treatments were very low (Table 5) because free fatty acids volatilizes from the oil under high temperature and vacuum deodorization conditions.

[0067] *Peroxide Value*. The peroxide values of all the treatments were below 1 meq/kg (Table 5) because of the vacuum conditions which limited oxidation. These measurements are within the acceptable limits of good quality edible oil.

[0068] *TAG Fatty Acid and CLA Isomer Analysis by FAMES GC-FID*. Table 8 below shows the total fatty acid composition produced in CLA-rich oil processed from the higher temperature-longer time study, respectively. A significant increase in palmitic acid was observed at temperatures $> 270^{\circ}\text{C}$. There were no significant differences in stearic acid content between treatments. There was a significant increase in oleic acid content at 282°C . There were significant reductions in linoleic and linolenic acid at temperatures $\geq 210^{\circ}\text{C}$.

Table 8: Total fatty acid composition of the processed oils obtained in an high temperature, long processing time study using a Central Composite Rotatable experimental design to optimize heterogeneous ruthenium catalysis of 250mL soy oil linoleic acid to CLA with 0.64% ruthenium catalyst over a range of temperatures (197-282 °C) and times (108-192 min).

Treatment	Time (min)	Temperature (°C)	Fatty Acid composition (%) of processed oils*						
			C16:0	C18:0	C18:1	C18:2 LA	C18:3	ct/nc CLA	tt CLA
1	180	210	14.84 _{bcd}	5.46 _a	27.40 _{ab}	33.80 _b	1.60 _{bc}	10.62 _{bc}	6.33 _{cd}
2	108	240	17.18 _{abc}	5.88 _a	29.38 _{ab}	25.88 _{cd}	0.60 _a	13.40 _a	7.65 _{bc}
3	180	270	18.09 _{ab}	6.86 _a	32.69 _{ab}	22.95 _{de}	0.27 _d	11.49 _{ab}	7.62 _{bc}
4	192	240	16.75 _{abcd}	6.16 _a	30.37 _{ab}	28.38 _c	0.76 _{cd}	9.00 _c	8.33 _{bc}
5	150	282	19.86 _a	7.87 _a	35.73 _a	17.82 _f	0.37 _d	10.55 _{bc}	7.78 _{bc}
6	150	240	16.67 _{abcd}	6.30 _a	29.49 _{ab}	20.82 _{ef}	0.44 _d	10.00 _{bc}	7.25 _{bcd}
7	120	210	13.30 _d	4.96 _b	25.70 _b	37.45 _{ab}	2.71 _a	8.35 _c	11.67 _a
8	150	197	13.48 _{cd}	5.06 _a	25.87 _b	38.06 _a	2.40 _{ab}	10.22 _{bc}	4.89 _d
9	150	240	16.20 _{abcd}	6.09 _a	29.80 _{ab}	29.37 _c	0.91 _{cd}	8.46 _c	8.88 _b
10	120	270	18.52 _{ab}	7.13 _a	33.50 _{ab}	22.98 _{de}	0.21 _d	8.79 _c	8.84 _b
* Means of respective fatty acid compositions in a column with same letter are not significantly different by Tukey's significance test.									

[0069] In this Example 5, it was found that the high temperature, long processing times study lead to a significant increase in oleic, stearic and palmitic acid composition and a significant decrease in linoleic acid and linolenic acid composition compared to the initial study with variable temperatures, processing times and catalyst concentration, with no significant increase in saturated fatty acids. The fatty acid composition of the oil was mainly affected by higher temperatures and longer processing times.

[0070] *CLA Isomers Determination by Silver-ion HPLC.* Silver-ion chromatography of CLA-rich soy oil produced at the optimized parameters showed 16 CLA isomers in 3 geometrical isomer groups (Table 9).

Table 9: Composition of CLA isomers in 21.05% CLA-rich oil obtained by processing RBD oil at 240 °C for 108 min with 0.64% ruthenium catalyst silver ion chromatography.

CLA isomers peak assignments	Composition (%)
<i>t-12,t-14</i>	1.98
<i>t-11,t-13</i>	7.00
<i>t-10,t-12</i>	18.70
<i>t-9,t-11</i>	17.42
<i>t-8,t-10</i>	4.51
<i>t-7,t-9</i>	0.49
<i>c-14,t-16/t-14,c-16</i>	0.64
<i>c-12,t-14/t-12,c-14</i>	1.35
<i>c-11,t-13/t-11,c-13</i>	12.02
<i>c-10,t-12/t-10,c-12</i>	14.11
<i>c-9,t-11/t-9,c-11</i>	6.11
<i>c-8,t-10/t-8,c-10</i>	3.35
<i>c-6,t-8/t-6,c-8</i>	9.75
<i>c-4,t-6/t-4,c-6</i>	0.26
<i>c-10,c-12</i>	0.30
<i>c-9,c-11</i>	2.00

[0071] The *trans,trans* CLA isomers comprised of 50% of total CLA. Of all *trans,trans* CLA isomers, *t-9,t-11* and *t-10,t-12* CLA were predominant and each comprised about 18%. The *trans,cis/cis,trans* CLA isomers constituted 47% of total CLA. The *cis,cis* CLA constituted 2% of total CLA. The first group consisted of 6 positional *trans,trans* CLA isomers, the second group of 8 positional *cis,trans/trans,cis* isomers and the last group of 2 positional *cis,cis* isomers. The control soy oil FAMES chromatogram did not show any CLA peaks because conjugated CLA was absent (data not shown).

EXAMPLE 6

[0072] The central composite rotatable experimental design of Example 6 decreased the catalyst concentration below 0.64% with the addition of formic acid and tocopherols in order to overcome the oil viscosity seen in Example 4. Example 6 used non-adsorbed unbleached soy oil that was only partially refined, *i.e.*, oil that had not been absorbed to remove residues from prior refining. Similar to prior examples, processing conditions were optimized to maximize CLA yields using central composite experimental design studies with variable time, catalyst concentration, formic acid concentration in both the oil and steam and variable tocopherol concentrations (Table 10 below). The process of Example 6 produced similar CLA yields to that obtained by fully refined soy oil used in the prior examples.

Table 10: Central composite rotatable design for Example 6 for optimization of 250mL soy oil linoleic acid to CLA over higher temperatures, longer processing times, and a range of ruthenium catalyst concentrations with the addition of formic acid to the oil and/or the steam.

Ru (%)	oil_FA (%)	Steam_FA (%)	Tocopherol (%)	Temp (°C)	Time (h)	CLA (%)
0	4	85	1	270	3	1.1
0.01	10	0	1	270	2	4.7
0.01	10	0	1	270	2	4.7
0.01	10	85	1	270	2	5.1
0.01	10	85	1	270	2	5.8
0.032	5	85	1	270	3	14.4
0.032	5	85	1	270	3	17.1
0.064	4	85	1	270	2	13.6
0.064	4	85	1	270	2	16.1
0.064	4	85	1	270	3	14.9
0.064	4	85	1	270	3	16.1
0.064	3	0	0	270	3	6.7
0.064	0	0	3	270	3	4
0.064	4	33	3	270	3	6.9
0.064	3	50	0	270	3	7.2
0.1	0	0	0	250	3	4.7
0.128	3	50	0	270	3	14

Ru (%)	oil_FA (%)	Steam_FA (%)	Tocopherol (%)	Temp (°C)	Time (h)	CLA (%)
0.128	4	33	0	270	3	15.5
0.128	0	33	0	270	3	6.7
0.128	4	33	1	270	3	17.8
0.128	0	33	3	270	3	5.7
0.128	4	33	1	280	2	14
0.128	4	33	3	270	3	12
0.128	0	33	3	270	3	5.7
0.128	4	85	1	270	3	22
0.128	4	85	1	270	3	23
0.128	4	33	1	270	5	20
0.128	4	33	1	270	4	14
0.128	4	85	1	270	2	24.8
0.128	4	85	1	270	2	23.5
0.128	4	85	1	270	3	26.6
0.128	4	85	1	270	3	20.5
0.128	4	33	1	270	3	13.7
0.128	4	50	1	270	3	25
0.128	4	33	1	270	2	13
0.128	5	85	1	240	2	22.6
0.128	5	85	1	240	2	16.8
0.128	4	85	1	270	3	21
0.128	4	85	1	270	3	24
0.128	4	85	1	270	3.5	20*
0.128	4	85	1	270	3.5	22*
0.128	4	50	1	270	3	12*
0.128	4**	99**	1	270	3	8**
* Unbleached oil						
** Acetic Acid						

[0073] The catalyst residue dissolved in the oil could subsequently be removed by the adsorption processing to reduce residual catalyst in the oil after being subjected to the process. For example, Ru catalyst levels should be <0.5ppm to be similar to Ni level allowed after hydrogenation. In addition, the adsorbent binding the Ru could possibly be used as a catalyst.

[0074] Formic acid is a volatile organic acid that was added to both the steam and the oil in Example 6 to promote Ru catalytic activity by Ru solubilization. In the low pressure system

of the present process, formic acid is lost rapidly and is added continuously with the steam. Alternatively, formic acid can be applied to the oil only during the beginning of the process to activate the catalyst, and subsequently applied with the steam for a predetermined amount of time, such as up to about 30 mins or between about 30 mins and about 60 mins.

[0075] In addition to formic acid, tocopherols were utilized in the processing of Example 6. One percent (1%) was previously found to be effective with iodine catalysis. Since tocopherols are a co-product of oil processing, there addition to the processing would neither be cost prohibitive nor need to be purchased for an industrial system.

[0076] Figure 3 illustrates the effect of ruthenium on the CLA yield at 270 °C for 3 hours with 4% formic acid in oil and steam of 85 % formic acid. Tables 11, 12 and 13 below illustrate the various effects of formic acid in oil and steam on CLA yield, and as can be seen, CLA yield increases 4.6 times in the presence of formic acid in oil, whereas steam formic acid results in less than 1 times increase.

Table 11: Effect of formic acid in oil and steam on CLA yield at 0.128% of ruthenium, at 270 °C for a 3 hrs reaction.

Formic acid in oil (%)	Steam formic acid (%)	CLA yield (%)
0	0	4.7 ± 0.5
0	33	5.7 ± 0.1
3	50	13.9 ± 0.2
4	33	14.5 ± 0.8
4	85	25.8 ± 1.1

Table 12: Effect of temperature on CLA yield at 0.128% of ruthenium, 4% formic acid in oil and 85% formic acid of steam for a 3 hrs reaction.

Temperature (°C)	CLA yield (%)
270	25.8 ± 1.1
240	19.4 ± 3.6

Table 13: Effect of reaction time on CLA yield at 270 °C, 0.128% of ruthenium, 4% formic acid in oil and 85% formic acid of steam.

Time (hours)	CLA yield (%)
2	24.1 ± 0.9
3	25.8 ± 1.1

EXAMPLE 7

[0077] The objective of Example 7 was to produce and characterize CLA-rich soy oil margarine relative to a soy oil control and commercial margarine. CLA-rich soy oil was used to prepare margarine. The samples were characterized for firmness, rheology, thermal behavior, solid fat content (SFC) and microstructure and compared with a soy oil control and commercial margarine. The CLA-rich oil margarine firmness and rheological properties were similar to commercial margarine and provided a better texture relative to the soy oil control margarine.

[0078] RBD soy oil was obtained from Riceland Foods (Stuttgart, AR, USA) and used throughout the study. Palm stearin, mono- and diacylglycerides were provided by Archer Daniels Midland (Decatur, IL, USA). Commercial Great Value margarine was purchased from Walmart store (Bentonville, AR, USA). Lecithin was purchased from Spectrum chemical manufacturing corporation (New Brunswick, NJ, USA). Other chemicals used were reagent grade.

[0079] *CLA-Rich Soy Oil Production and Fatty Acid Analysis.* CLA-rich soy oil was produced by the heterogeneous catalytic method of the prior examples discussed above. The fatty acid composition of soy and CLA-rich oil were determined in triplicate using the base catalyzed derivatization technique to produce FAME and subsequent GC-FID analysis.

[0080] *Margarine Preparation.* Optimization of hard-stock content in fat phase for margarine production Hardstock optimization was determined with 30, 40, 50 % palm stearin (hard stock) in 18.85 % CLA-rich soy oil in duplicate. These blends were used to produce

margarines in duplicate. The oil phase consisted of (w/w, %): 79.37 % fat, 0.42 % mono- and diacylglycerides, 0.2 % soy lecithin and 0.0048 % beta-carotene. The aqueous phase consisted of (w/w, %): 17.65 % distilled water and 2.25 % table salt. Both phases were vigorously mixed for 10 min using a Black & Decker MX300 homogenizer (New Britain, CT, USA) for emulsification. The resulting emulsion was then crystallized using a half-pint Hamilton Beach ice-cream maker (Glen Allen, VA, USA). The bowl was maintained at around 10 °C during the crystallization. The resulting crystallized emulsion was then tempered at room temperature for 4 h and then worked vigorously with a hand mixer to obtain a smooth and consistent texture. The resulting paste-type margarine samples (300 g each) were placed in sealed tubs and stored at 5 °C prior to analysis. Microscopic analysis showed that the margarine prepared with 50 % palm stearin (hard stock) had least air incorporation and was used in subsequent studies.

[0081] *Margarine production for characterization.* The 50 % palm oil stearin (hardstock) was used throughout in this Example 7. CLA-rich soy oil margarine and a control margarine prepared with conventional soy oil were produced in duplicates.

[0082] *Margarine Characterization.* CLA-rich soy oil margarine and soy oil control margarines were compared with a commercial Great Value margarine (Walmart, Bentonville, AR, USA).

[0083] *Firmness Analysis.* Measurements were performed on five replicates selected randomly from the two margarine batches. Samples were placed in 5 cm x 3 cm plastic cups and stored at 5 °C in a thermostatic cabinet for an hour before analysis. Firmness was determined as force required for penetration using a 5942 Instron TA 500 Texture Analyzer (Lloyd Instrument, Bognor Regis, West Sussex, UK). A 11 mm diameter cylindrical probe penetrated the sample to a depth of 10 mm at a rate of 10 mm/min with 0.1 N trigger value at 5 °C.

[0084] *Rheology.* The rheology was determined in triplicate for all margarine samples. An advanced rheometer AR 2000 (TA Instruments, New Castle, DE, USA) using a parallel plate with a cross hatched geometry (diameter 40 mm) was used. The geometry gap of the rheometer was set at 1,000 μm . Oscillatory stress sweeps were carried out to gain insights into the rheological behavior of margarine samples. Oscillation procedure was performed in three steps: Firstly, a conditioning step at 5 °C as initial temperature for a duration of 15 min followed by a stress sweep from 0.1 to 10,000 (Pa) to determine the linear viscoelastic region at a frequency of 1 Hz. Finally, a postexperiment step was performed at 5 °C. Complex modulus (G^*) and phase angle (δ) were measured as a function of oscillatory stress. G^* is the measure of the total resistance of sample to deformation and phase angle (which is a ratio of loss and storage moduli) is a measure of relative viscous or elastic behavior of the sample. The phase angle values indicate the degree of viscosity and elasticity. Small angles indicate solid-like behavior, with increasing angles indicative of an increasingly liquid behavior.

[0085] *Thermal Behavior.* The DSC experiments were performed with a Q1000 Tzero DSC (TA Instruments, New Castle, DE, USA) on triplicate margarine samples. The DSC was calibrated with indium (enthalpy and temperature), azobenzene (temperature) and undecane (temperature) before analyses. Nitrogen was used to purge the system and an empty pan was used as a reference. The margarines were sampled (5–10 mg) in hermetic aluminum pans and sealed. The melting profile was recorded by applying a time–temperature program as follows: initial temperature at 5 °C (followed by loading of cup), holding for 5 min and heating at 5 °C/min to 80 °C. The integration of obtained melting curves was performed using the Universal Analysis software (TA Instruments, New Castle, USA).

[0086] *Solid Fat Content (SFC) Determination.* SFC-profile was measured by 23 MHz ¹H NMR Maran instrument (Oxford Instruments, Oxfordshire, UK) in triplicate. Prior to analysis, the fat phases of the margarines were obtained after destabilization at 65 °C followed by filtration over sodium sulfate. Molten fat was placed in NMR tubes and submitted to the temperature treatments of the official method AOCS Cd 16b-93. The SFC was determined from 5 °C at 5 °C intervals until completely melted following 30 min incubations at each specific temperature.

[0087] *Microstructure.* Microscopic analyses were conducted by the use of a Leitz Diaplan microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) equipped with a Linkam PE 94 temperature control system (Linkam, Surrey, Germany) in triplicate. Since, the undiluted samples were too dense to be viewed clearly under the microscope; slide preparation was done by smearing the sample on the slide followed by dilution with a drop of oil for better visual assessment. The slides were placed on the temperature controlled plate to visualize the microstructure under transmitted polarized and normal light at 20 °C. Images were recorded with an Olympus' Color View camera and processed with Cell D software (Olympus, Aartselaar, Belgium).

[0088] *Droplet Size Distribution.* Triplicate water droplet size analysis of the samples was performed by pulsed field gradient nuclear magnetic resonance (pfg-NMR) on a bench-top Maran Ultra spectrometer (Oxford Instruments, UK) operating at a frequency of 23.4 MHz in combination with the droplet size application. Samples were analyzed at 5 °C to minimize inter-droplet water diffusion. To suppress the NMR contribution of the fat phase, pfg-NMR experiments were conducted using an inversion recovery stimulated echo pulse sequence. In the performed experiments, the diffusion time (Δ) was set to 0.2 s; the gradient strength was fixed at

1.74 T/m, while the gradient duration (δ) was varied in 17 steps from 400 to 4,500 ms. By measuring the echo attenuation ratio of the NMR signal as a function of the gradient duration, it is possible to determine the hindered diffusion behavior and hence the droplet size distribution. Polydispersity index (PDI) was then calculated from the mean and standard deviation values. It is the measure of non-uniformity of water droplet size distribution in the margarine.

[0089] *Statistical Analysis.* JMP 10 (SAS Institute Inc., Cary, NC) was used to perform analysis of variance (ANOVA) and Student's *t* test to compare means ($P < 0.05$).

[0090] *CLA-Rich Oil Fatty Acid Analysis.* Table 14 below shows the fatty acid composition of CLA-rich soy oil and conventional soy oil, used to produce CLA-rich soy oil and control soy oil margarines. The soy oil has a typical soy oil fatty acid profile with 55.86 % linoleic acid as a dominant fatty acid. However, CLA-rich soy oil comprises of 13.25 % *cis,trans/trans,cis* CLA isomers and 5.6 % are *trans,trans* CLA isomers to produce a total of 18.85 % CLA-rich soy oil, with a 35.71 % reduction in linoleic acid. About 16.8 g of the CLA-rich soy oil will provide 3.2 g CLA that is required to provide clinical benefits.

Table 14: Fatty acid composition of soy oil and CLA-rich soy oil measured in triplicate as GC-FID FAME.

Fatty acid	Soy oil (%)	CLA-rich soy oil (%)
Palmitic acid	13.48 $\pm$ 0.14	13.27 $\pm$ 0.10
Stearic acid	4.68 $\pm$ 0.12	4.98 $\pm$ 0.06
Oleic acid	24.11 $\pm$ 0.22	24.24 $\pm$ 0.26
Linoleic acid	55.86 $\pm$ 0.24	35.71 $\pm$ 0.86
Linolenic acid	4.84 $\pm$ 0.11	1.62 $\pm$ 0.03
<i>trans,cis/cis,trans</i> CLA	-	13.25 $\pm$ 0.12
<i>trans,trans</i> CLA	-	5.60 $\pm$ 0.03

[0091] *Firmness Analysis.* Figure 4 shows the comparison plot of average force measured versus compressive extension for CLA-rich oil margarine, control soy oil margarine and commercial margarine. The results were reproducible, as the standard deviations were within 5 % of the mean force values for each margarine sample. The CLA-rich soy oil and control soy oil margarine load curves were significantly different from that of commercial margarine, with absence of a defined fracture force indicating a more plastic structure. However, the hardness of CLA-rich soy oil margarine was significantly greater than that of the control soy oil margarine ($P<0.05$) and similar to the commercial margarine. Hardness is one of the most important macrostructural properties of fat systems and is widely used to characterize margarine functionality. Therefore, CLA-rich oil may contribute to the hardness that consumer desire in a commercial margarine, possibly by strengthening the lipid crystal network. This could be possibly due to the presence of linear *trans,trans* CLA fatty acids which are more readily incorporated than LA into crystal networks.

[0092] The firmness of CLA-rich soy oil margarine and commercial margarine are quite similar. However, the commercial margarine force-extension curve differed by having a prominent fracture force followed by a decline in slope of load curve. This indicates that the commercial margarine has certain fracturability leading to a breakdown in structure as the deformation is induced, which is not present in the others margarine curves.

[0093] *Rheology.* Figure 5 shows the complex modulus (G^*) versus oscillatory stress relationship for each margarine. The linear viscoelastic region was narrower for control soy oil margarine (1–10 Pa) relative to CLA-rich soy oil margarine and commercial margarine (10–1,000 Pa). The critical value of oscillatory stress (<100 Pa) required to deform the soy oil margarine structure was smaller than that for CLA-rich soy oil and commercial margarine

(>1,000 Pa). This means that the microstructure of CLA-rich soy oil and commercial margarine were more resistant to deformation under oscillatory stress, than the control soy oil margarine. The CLA-rich soy oil and commercial margarine rheology curves are similar and are consistent with the similar firmness measurements for these samples.

[0094] Figure 6 shows the plots of phase angle with applied oscillatory stress. The results obtained from CLA-rich soy oil margarine and commercial margarines were comparable. There was a gradual increase in the phase angle (5–15°) until oscillatory stress of around 5,000 Pa. However, on further additional stress there was a sudden increase in the phase angle to 35°, indicating that both CLA-rich soy oil margarine and commercial margarine maintain their elastic solid structure even at fairly high stress amplitude of 8,000 Pa. In contrast, control soy oil margarine showed a gradual increase in the phase angle from 10 to 20° up to 400 Pa of oscillatory stress. On further increase, in amplitude of stress, the phase angle suddenly increased to 80°, indicating rapid transformation to a much more viscous liquid-like substance. This suggests that control soy oil margarine has less elastic fat behavior at any given oscillatory stress than CLA-rich soy oil and commercial margarine. Therefore, larger amplitude of oscillatory stress is required to transform the elastic solid character of CLA-rich soy oil and commercial margarine to a viscous liquid.

[0095] *Thermal Behavior.* Figure 7 shows the melting behavior of margarine samples monitored by differential scanning calorimetry. The results were highly reproducible as the triplicate curves from each replication were identical. The melting profiles of CLA-rich oil and soy oil margarines were similar. Both samples have a very prominent melting fraction around about 58 °C; 57.6 °C for CLA-rich soy oil margarine and 58.2 °C for the control soy oil margarine. This similarity is to be expected since the same SFC was used. However, the

commercial margarine showed two broad, shallow peaks at 13.3 and 29 °C. The melting profile of commercial margarine was very different, indicating a different formulation than the other samples. Despite of the similar melting profiles of the CLA-rich soy oil and control soy oil margarines, CLA-rich soy oil margarine made much better contribution to firmness and rheological properties that closely resembled the commercial product. This suggests that the CLA components in the soy oil significantly contributed to margarine texture, which could be related to the *trans,trans* CLA content.

[0096] *SFC Determination.* Figure 8 shows the SFC curves of control soy oil margarine, CLA-rich soy oil margarine and commercial margarine as a function of temperature. Commercial margarine had significantly lower SFC as compared to control soy oil and CLA-rich soy oil margarines at all temperatures ($P < 0.05$). CLA-rich oil and control soy oil margarines were similar and had a more gradual decrease of SFC with increase in temperature than the commercial margarine. These results are consistent with the corresponding DSC melting profiles.

[0097] There is a slight, but significant, difference between the CLA and soy oil control margarine samples between 5 and 15 °C. CLA-rich oil has slightly but significantly greater SFC in that range of temperatures (5–15 °C) ($P < 0.05$). This could partly explain the increased hardness (see Figure 4) and significantly different rheological properties (Figures 5 and 6), which were both measured at 5 °C. However, at temperatures over 15 °C there was no significant difference in SFC content between the soy oil and CLA-rich oil margarine ($P > 0.05$). This data shows that SFC and melting profile are not the sole factors influencing the texture and strength of a fat crystal network and indicate that presence of CLA in margarine may contribute to the hardness. However, SFC and hardness do not always have a linear correlation.

[0098] *Microstructure.* Microscopic images of control soy oil margarine, CLA-rich soy oil margarine and commercial margarine are shown in Figure 9. There was significant air-incorporation in the control soy oil and CLA-rich soy oil margarine, as shown by the thick, black outlined circles in normal light microscopy, which were minimal in commercial margarine. The size of water droplets, seen as white solid particles in polarized light microscopy, were larger in CLA-rich soy oil and control soy oil margarine ($r = 8\text{--}20\text{ }\mu\text{m}$) than the commercial margarine ($r < 5\text{ }\mu\text{m}$).

[0099] *Droplet Size Distribution.* Table 15 below shows the average volume weighted water droplet size and PDI of the control soy oil margarine, CLA-rich soy oil margarine and commercial margarine. The water droplet size of commercial margarine is approximately five- to sixfold smaller than control soy oil and CLA-rich soy oil margarine. As seen from Table 15, the control soy oil margarine has highest PDI (0.12), followed by CLA-rich soy oil margarine (0.06), while commercial margarine has least PDI (0.01). The droplet size distribution data corresponds with the microstructure shown in Figure 9.

Table 15: Water droplet size and polydispersity index of control soy oil margarine, CLA-rich soy oil margarine and commercial margarine.

	Volume mean weighted radius (μm)	Polydispersity index
Soy oil margarine	11.95 ± 1.53	0.12
CLA-rich oil margarine	9.42 ± 0.54	0.06
Commercial margarine	1.89 ± 0.01	0.01

[0100] The better water droplet distribution and smaller water droplet size of the commercial margarine reflects the improved industrial preparation techniques, homogenization and scraped surface heat exchangers, used at commercial scale than the samples produced with the hand blender and ice-cream maker used to prepare experimental margarines.

[0101] As can be concluded from Example 7, CLA-rich soy oil provides an improved margarine texture than control soy oil. The CLA-rich soy oil margarine hardness and rheological properties obtained at 5 °C were comparable to commercial margarine but SFC, droplet size distribution and melting behavior of CLA-rich oil margarine were similar to control soy oil margarine, except at 5–15 °C, which explains firmness and rheology differences. This suggests that hardness and rheological properties of margarine are not solely dependent on SFC and melting behavior. Lipid composition, polymorphism and microstructure differences in CLA-rich soy oil relative to the control soy oil may play an important role on the texture and rheological properties of margarine.

[0102] A 7 g typical serving of the experimental CLA-rich oil margarine will provide 0.6 g CLA. Thus five servings will provide about 3.2 g/day of CLA and 185 calories/day, which is well within the maximum recommended 700–980 fat calories/day. However, there was more hard fat in the Example 7 samples than would be used in a commercial product. Therefore, industrial production would be able to increase the CLA content in a serving at the expense of hard fat due to improved processing.

EXAMPLE 8

[0103] The objective of Example 8 was to produce and characterize CLA-rich soy oil shortening relative to a commercial shortening, namely Crisco®. Similar to Example 7 for margarine above, CLA-rich soy oil was used to prepare shortening this Example 8. The samples were characterized for hardness using a puncture force test compared with a commercial shortening. The CLA-rich oil shortening hardness was similar to commercial shortening.

[0104] As shown in Table 16, for the method of Example 8, palm stearin and soy oil were heated to 70-77 °C while stirring constantly. The mixture was poured into a stainless-steel

beaker and immediately placed in 10 °C water bath while stirring constantly with a hand-mixer on the lowest speed setting. The mixture was cooled to a predetermine cooling temperature, namely, 21 °C, 18 °C or 15 °C. The resulting shortening was placed in plastic centrifuge tubes and stored at room temperature for at least one week to temper. Puncture force tests were carried out to explore CLA ranges and harnesses, and were performed with TA-XT2i texture analyzer 2mm diameter cylinder probe for CLA shortening formulation and Crisco[®], a commercial shortening.

Table 16: Shortening Formulations.

Sample #	% CLARSO*	% Stearin	Cooling Temp (°C)
1	30	70	21
2	35	65	21
3	40	60	21
4	45	55	21
5	50	50	21
6	30	70	18
7	35	65	18
8	40	60	18
9	45	55	18
10	50	50	18
11	30	70	15
12	35	65	15
13	40	60	15
14	45	55	15
15	50	50	15
* CLA-Rich Soy Oil			

[0105] As shown in Figure 10, the commercial shortening had a puncture force of 0.5 N. Using regression analysis, best-fit line for samples cooled to each of the three temperatures was determined: 21°C: $y = -0.1108x + 7.8418$, with the intersection with commercial control at $x = 66.0\%$ CLARSO; 18°C: $y = -0.0442x + 4.0194$, with the intersection with commercial control at $x = 79.0\%$ CLARSO; and 15°C: $y = -0.0265x + 2.9368$, with the intersection with commercial control at $x = 90.9\%$ CLARSO. Based on this, the ideal CLA shortening formulations to make a

shortening product having similar hardness properties to the commercial control, Crisco[®], are below in Table 17.

Table 17: Ideal CLA shortening formulations to make 'Crisco identical' (0.5 N) product.

% CLARSO	% Stearin	Cooling Temp (°C)
66	34	21
79	21	18
91	9	15

[0106] In conclusion, the shortening formulations of this Example 8 gave the same puncture force as the commercial control shortening.

[0107] Whereas, the processes and compositions have been described in relation to the drawings and claims, it should be understood that other and further modifications, apart from those shown or suggested herein, may be made within the scope of this invention.

WHAT IS CLAIMED IS:

- 1 1. A process for producing conjugated linoleic acid-rich oil, said process comprising the
2 steps of:
3 heterogeneously catalyzing a linoleic acid-rich oil and a catalytic amount of a
4 transition metal having a vacant 3d- or 4d-orbital to produce said conjugated linoleic
5 acid-rich oil.
- 1 2. The process of Claim 1 further comprising the step of extracting said transition metal
2 from said conjugated linoleic acid-rich oil.
- 1 3. The process of Claim 1 wherein said linoleic acid-rich oil is a triacylglyceride vegetable
2 oil.
- 1 4. The process of Claim 3 wherein said triacylglyceride vegetable oil is selected from the
2 group consisting of soy, sunflower, corn, cottonseed or peanut oil.
- 1 5. The process of Claim 1 wherein said transition metal is selected from the group
2 consisting of ruthenium, rhodium, molybdenum, palladium, gold, silver, copper, iron, manganese
3 or nickel.
- 1 6. The process of Claim 5 wherein said transition metal is ruthenium or nickel.
- 1 7. The process of Claim 1 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal in pressure conditions between approximately 1

3 mm Hg and approximately 2 mm Hg for between approximately 108 minutes and approximately
4 240 minutes to produce said conjugated linoleic acid-rich oil.

1 8. The process of Claim 7 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal at temperature conditions between approximately
3 165 °C and approximately 282 °C.

1 9. The process of Claim 1 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal at substantially vacuum pressure for up to
3 approximately 240 minutes to produce said conjugated linoleic acid-rich oil.

1 10. The process of Claim 9 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal at temperature conditions between approximately
3 165 °C and approximately 282 °C for between approximately 60 minutes and approximately 240
4 minutes to produce said conjugated linoleic acid-rich oil.

1 11. The process of Claim 10 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal between approximately 1 mm Hg and
3 approximately 2 mm Hg at a temperature of between approximately 210 °C and approximately
4 270 °C for between approximately 120 minutes and approximately 180 minutes.

1 12. The process of Claim 10 wherein said catalytic amount of said transition metal comprises
2 between 0.08% to approximately 8% of said transition metal.

1 13. The process of Claim 12 wherein said catalytic amount of said transition metal is between
2 approximately 0.08% to approximately 0.75% of a ruthenium catalyst or approximately 8% of a
3 nickel catalyst.

1 14. The process of Claim 1 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal in pressure conditions between approximately 1
3 mm Hg and approximately 2 mm Hg and temperature conditions between approximately 165 °C
4 and approximately 282 °C to produce said conjugated linoleic acid-rich oil.

1 15. The process of Claim 14 wherein said catalysis step further comprises catalyzing said
2 linoleic acid-rich oil and said transition metal for between approximately 60 minutes and
3 approximately 240 minutes.

1 16. The process of Claim 15 wherein said catalysis step further comprises heterogeneous
2 catalyzing via vacuum distillation of said linoleic acid-rich oil and said transition metal at a
3 temperature of between approximately 210 °C and approximately 270 °C and at a substantially
4 vacuum pressure for between approximately 120 minutes and approximately 180 minutes.

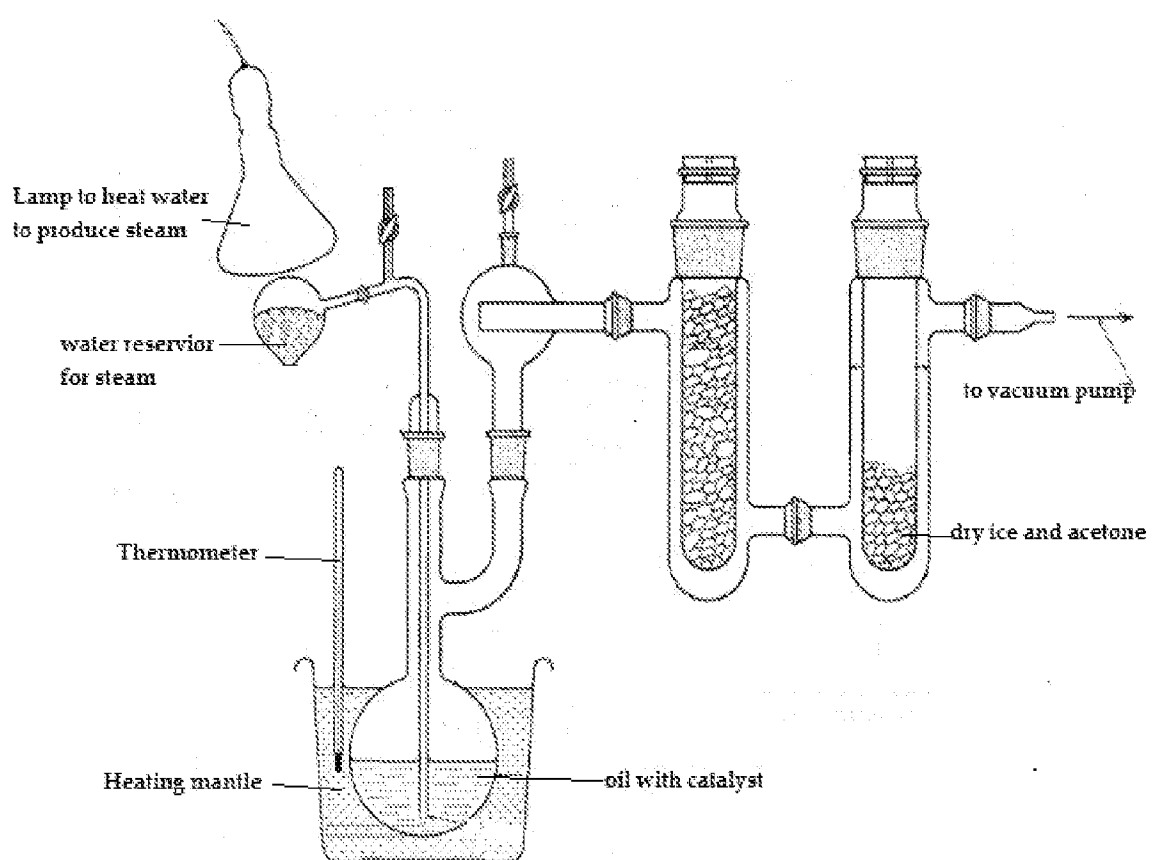
1 17. The process of Claim 14 wherein said catalytic amount of said transition metal comprises
2 between approximately 0.08% and approximately 8% of said transition metal.

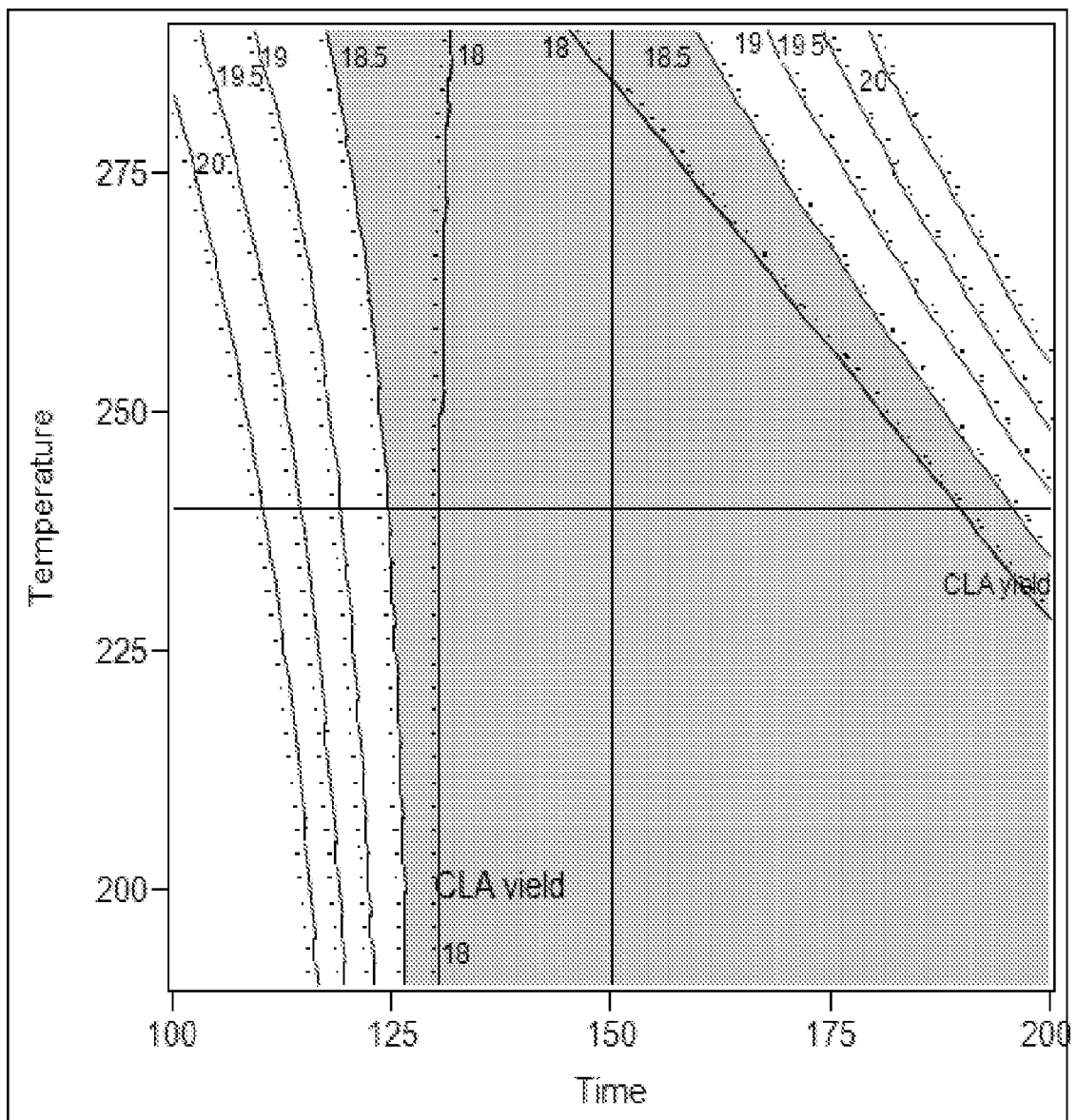
1 18. The process of Claim 17 wherein said catalytic amount of said transition metal is between
2 approximately 0.08% to approximately 0.75% of a ruthenium catalyst or approximately 8% of a
3 nickel catalyst.

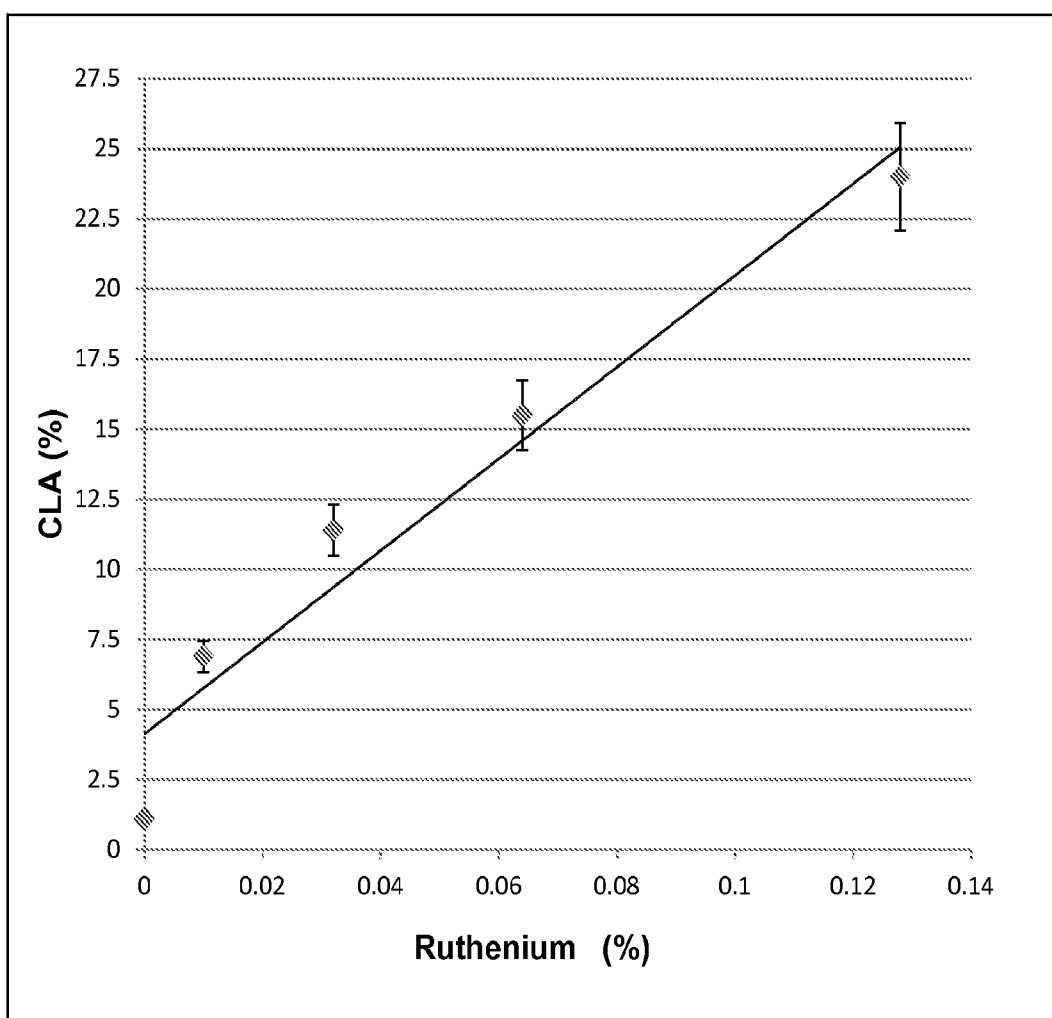
1 19. The process of Claim 1 wherein said catalytic amount of said transition metal comprises
2 between 0.08% to approximately 8% of said transition metal.

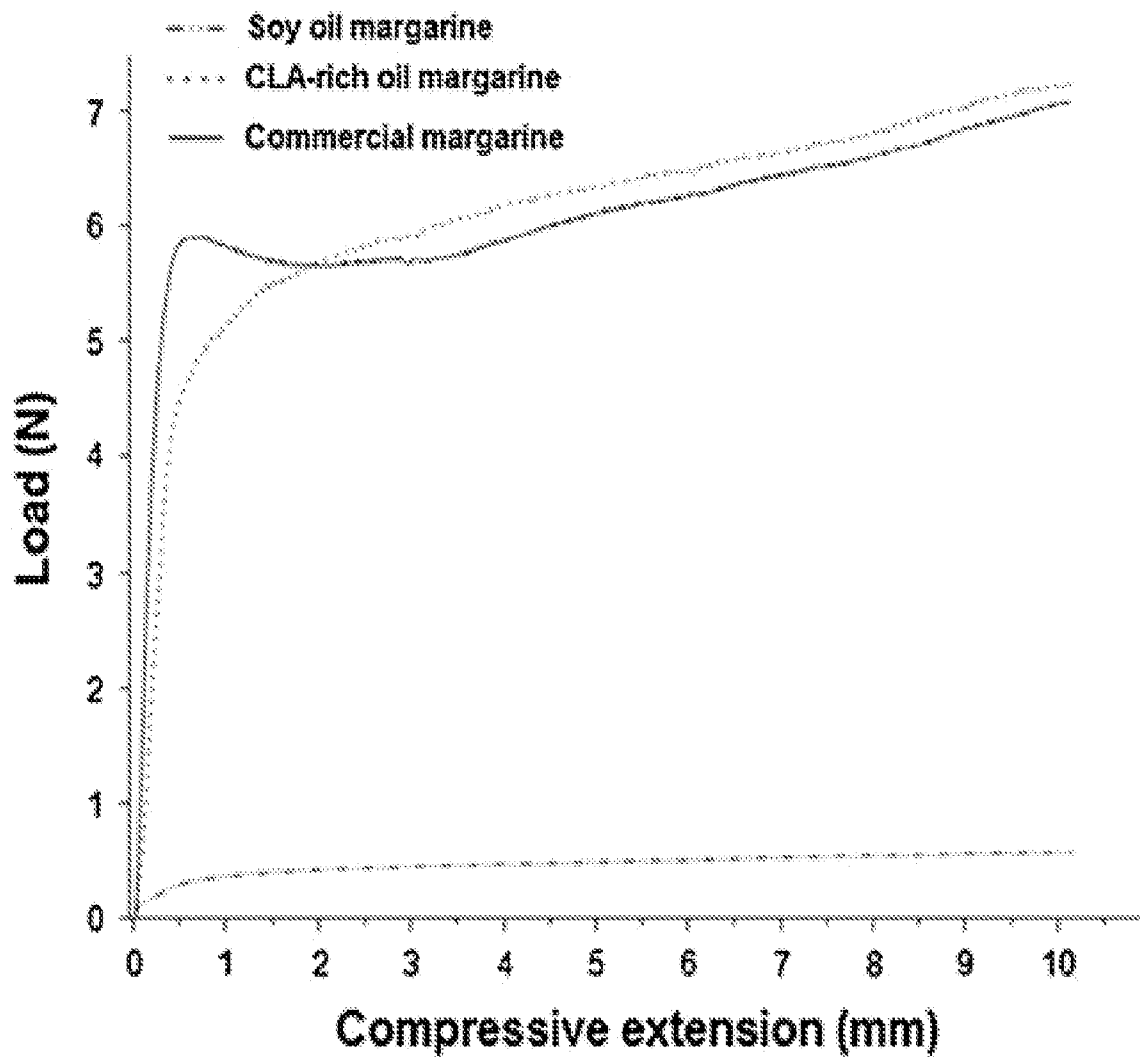
1 20. The process of Claim 19 wherein said catalytic amount of said transition metal is between
2 approximately 0.08% to approximately 0.75% of a ruthenium catalyst or approximately 8% of a
3 nickel catalyst.

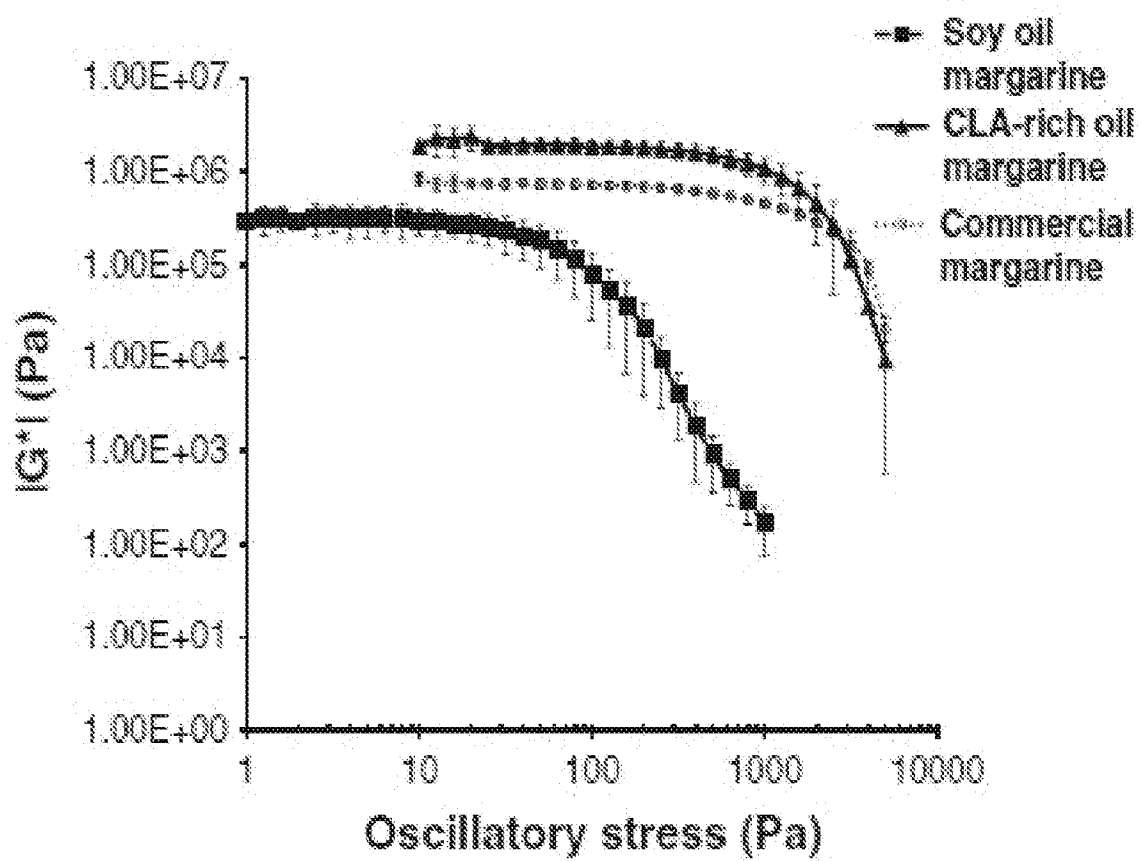
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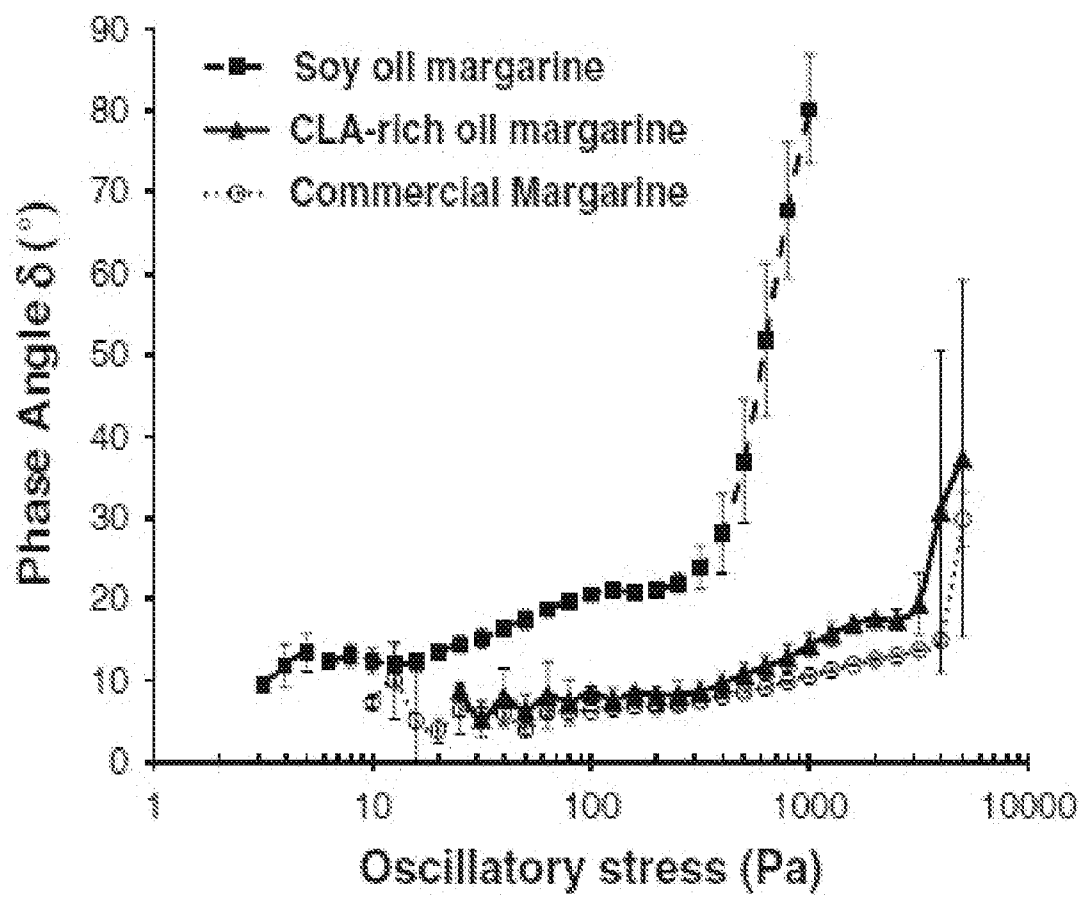
**FIG. 1**

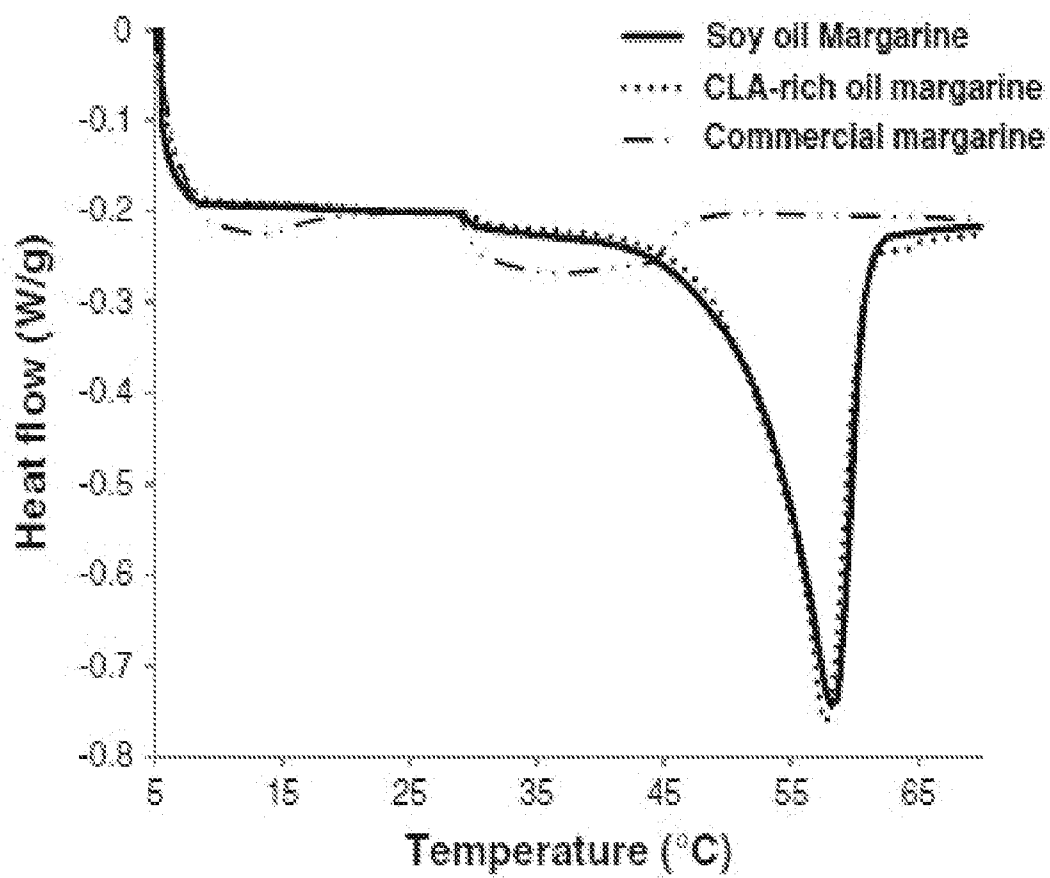
**FIG. 2**

**FIG. 3**

*FIG. 4*

**FIG. 5**

**FIG. 6**

**FIG. 7**

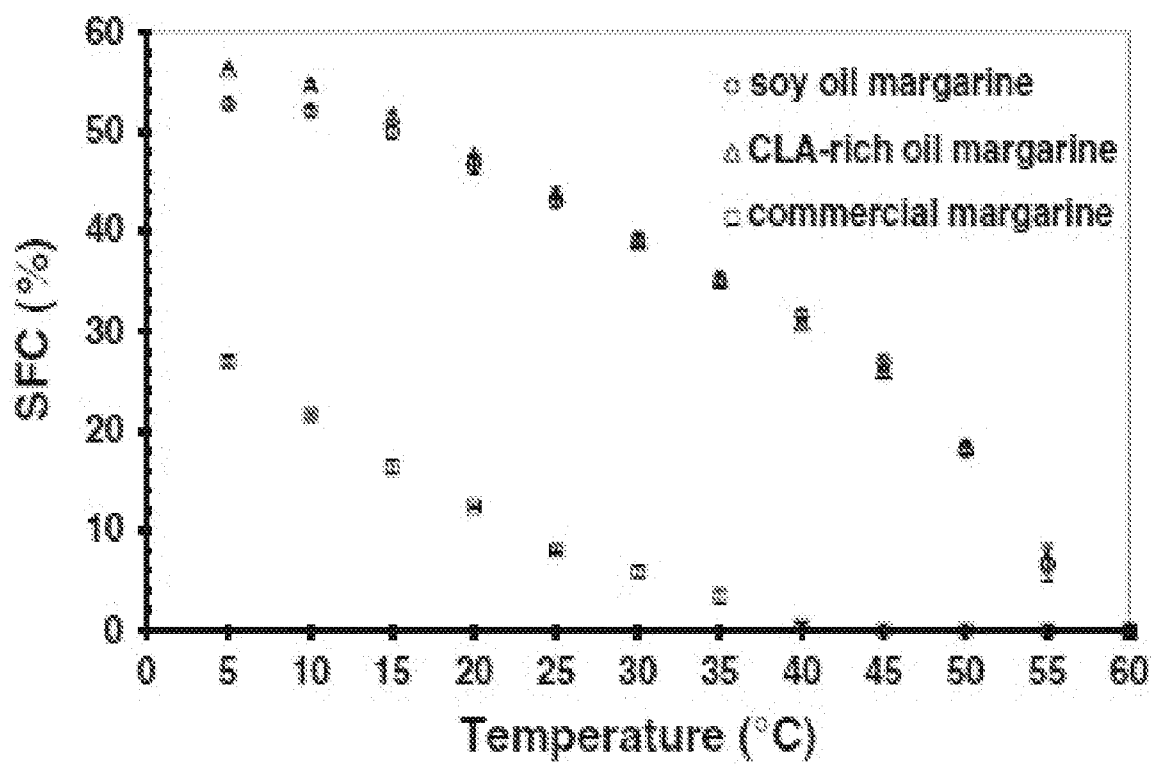
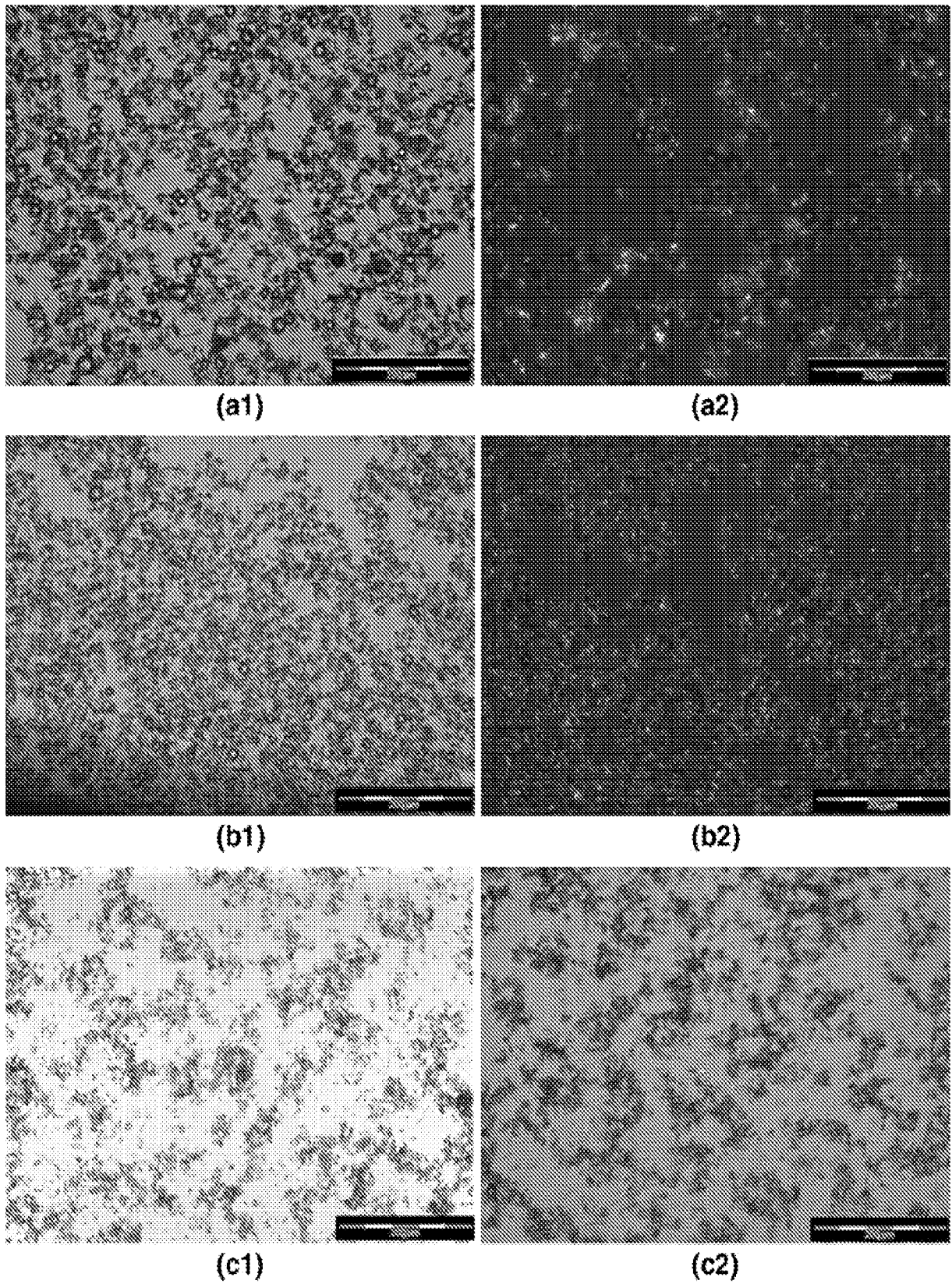
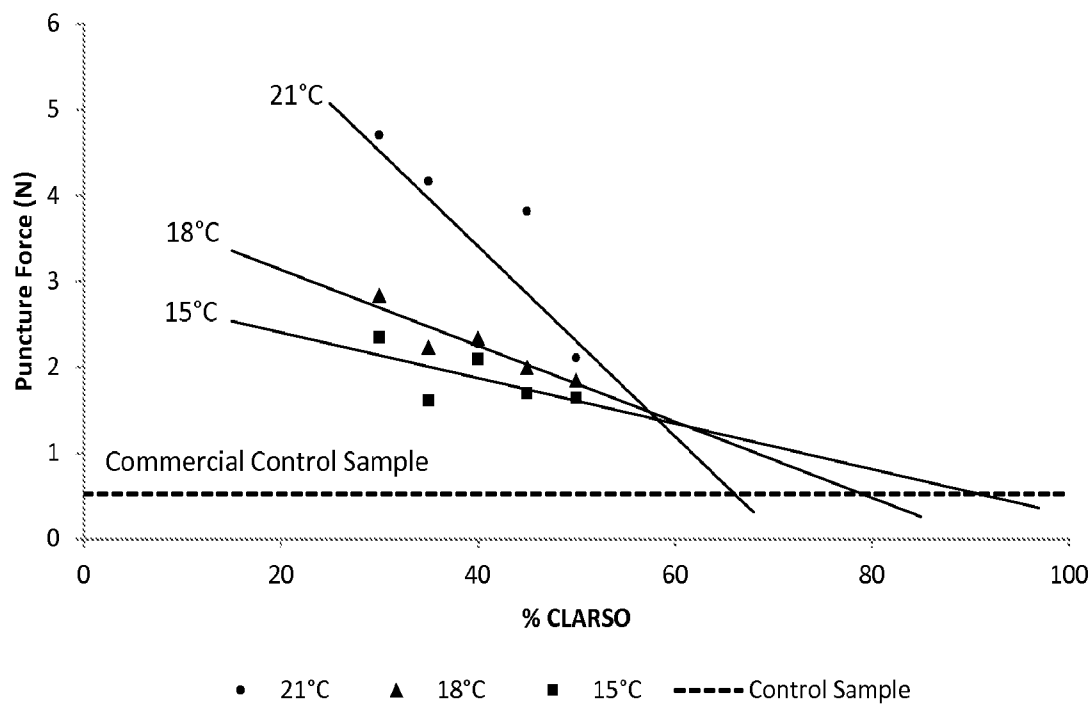


FIG. 8

**FIG. 9**

**FIG. 10**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/15139

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C11C 3/14; C07C 51/347, 51/353 (2014.01) USPC - 554/125, 126 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): C11C 3/14; C07C 51/347, 51/353; B01J 29/12, 29/44, 29/74 (2014.01) USPC: 554/125, 126 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; PubMed; IP.com; University of Arkansas, Proctor, Shah, conjugated linoleic acid, linoleic acid-rich oil, transition metal, ruthenium, nickel, percent, heterogeneous, catalysis, soy, sunflower, corn, cottonseed, peanut, mm Hg, atm, minute, temperature, vacuum distillation, isomerization																				
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X --- Y</td> <td>WO 2012/068645 A2 (GEBOERS, J et al.) May 31, 2012; abstract; page 6, lines 15-20; page 13, lines 24-35; page 14, lines 1-5; page 20, lines 1-5; page 23, lines 15-23; page 32, lines 8-10</td> <td>1-6, 19-20 --- 12-13, 17-18</td> </tr> <tr> <td>X --- Y</td> <td>US 6,380,409 B1 (SAEBO, A et al.) April 30, 2002; column 6, lines 10-15; column 8, lines 33-36; column 10, lines 1-65, examples 1-7; column 11, lines 25-30; column 15, lines 35-38</td> <td>1, 7-11, 14-16 --- 12-13, 17-18</td> </tr> <tr> <td>A</td> <td>US 4,203,911 A (SCARPIELLO, DA) May 20, 1980; entire publication</td> <td>1-20</td> </tr> <tr> <td>A</td> <td>US 7,417,159 B2 (GALVEZ, JMG et al.) August 26, 2008; entire publication</td> <td>1-20</td> </tr> <tr> <td>A</td> <td>US 2007/0191619 A1 (RONGIONE, JC et al.) August 16, 2007; entire publication</td> <td>1-20</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X --- Y	WO 2012/068645 A2 (GEBOERS, J et al.) May 31, 2012; abstract; page 6, lines 15-20; page 13, lines 24-35; page 14, lines 1-5; page 20, lines 1-5; page 23, lines 15-23; page 32, lines 8-10	1-6, 19-20 --- 12-13, 17-18	X --- Y	US 6,380,409 B1 (SAEBO, A et al.) April 30, 2002; column 6, lines 10-15; column 8, lines 33-36; column 10, lines 1-65, examples 1-7; column 11, lines 25-30; column 15, lines 35-38	1, 7-11, 14-16 --- 12-13, 17-18	A	US 4,203,911 A (SCARPIELLO, DA) May 20, 1980; entire publication	1-20	A	US 7,417,159 B2 (GALVEZ, JMG et al.) August 26, 2008; entire publication	1-20	A	US 2007/0191619 A1 (RONGIONE, JC et al.) August 16, 2007; entire publication	1-20
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A	US 2007/0191619 A1 (RONGIONE, JC et al.) August 16, 2007; entire publication	1-20																		
☐ Further documents are listed in the continuation of Box C. ☐																				
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Date of the actual completion of the international search 16 April 2014 (16.04.2014)		Date of mailing of the international search report 07 MAY 2014																		
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