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Effects of Rhodium Heterogeneous Catalyst and Isomerization Conditions on Linoleic Acid Conjugation of Soybean Oil

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Rhodium heterogeneous catalyst was used to catalyze isomerization of linoleic acid in soybean oil to conjugated linoleic acid (CLA). A central composite rotatable design with five levels of three variables, namely, reaction temperature, stirring speed, and reaction time, was used to determine the maximum CLA yield. The formation of CLA during isomerization was greatly dependent on the reaction temperature and time. The CLA content of soybean oil increased from 0.63 to 202.42 mg/g oil when isomerization was done at 200 °C, with a stirring speed of 200 rpm for 49 min. Analysis of triacylglycerol positions showed that linoleic acid at any position in a triacylglyceride could possibly be isomerized to CLA.

KEYWORDS: CLA; conjugated linoleic acid; rhodium heterogeneous catalyst; soybean oil

INTRODUCTION

Conjugated linoleic acid [CLA; $CH_3(CH_2)_5CH{=}CHCH{=}CH(CH_2)_7COOH$] refers to a mixture of positional and geometrical isomers of linoleic acid with conjugated double bonds. It is found predominantly in meat and dairy products due to isomerization of linoleic acid to CLA in ruminant animal by Gram-positive bacteria such as *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, and *Eubacterium* sp. (*1*). It has been reported that health benefits of CLA may include anticarcinogenesis (*2*−*4*), antiatherosclerosis (*5*), enhancing immune function (*6*), and body fat reduction (*7*, *8*). Commercially, CLA is mainly produced by alkaline isomerization of linoleic acid. This isomerization method has been known as diene conjugation since 1951 (*9*) in which 9*c*,11*t*-18:2 and 10*t*,12*c*-18:2 fatty acids are predominant isomers from the synthesis. However, using an excess of strong basic potassium hydroxide or sodium methoxide is disadvantageous (*10*). Furthermore, CLA in its chemical form of a free fatty acid (FFA) during alkaline isomerization is easily oxidized in air (*11*). Other methods for conjugating diene stuctures include using homogeneous and heterogeneous catalysts. Homogeneous transition metal catalysts such as $RhCL(PPh_3)_3$, $[RhCl(C_8H_{14})_2]_2$, and $RhCl{\cdot}2H_2O$, have been used to study the isomerization of double bonds in organic compounds including linoleic acid (*12*, *13*). However, it is difficult to separate soluble homogeneous catalysts from the final product.

Heterogeneous catalysts used for double bond hydrogenation not only facilitate hydrogenation but also have the tendency to catalyze isomerizations and double bond migrations (*14*). Mossoba et al. (*15*) and Jung et al. (*16*) showed the potential of conjugation of unsaturated fatty acid in edible oil using a heterogeneous nickel catalyst. Palladium, platinum, and rhodium are also active catalysts for hydrogenation and possible isomerization. In addition, they are easy to use and separate from the isomerized oil product. From our preliminary studies on the potential use of palladium, platinum, and rhodium catalysts for the conjugation of fatty acids, rhodium was shown to be the most effective catalyst for conjugation of diene at high temperature and ethylene glycol was a good medium in the reaction.

This paper presents a study of the effect of isomerization conditions using a heterogeneous catalyst (Rh on carbon) on the formation of CLA in soybean oil. Ag^+ high-performance liquid chromatography (HPLC), gas chromatography (GC), and GC-MS were used to determine and identify the CLA isomers. Triacyl-*sn*-glycerols in isomerized soybean oil were partially hydrolyzed with pancreatic lipase and analyzed by thin-layer chromatography (TLC) and Ag^+ HPLC for determining CLA position in the triacyl-*sn*-glycerols.

MATERIALS AND METHODS

Materials. Soybean oil was purchased from Wal*Mart Stores Inc. (Bentonville, AR, Lot 030203CAA). Three standard CLA methyl ester (CLAME) isomers [9*c*,11*c*; 9*c*,11*t*; and 9*t*,11*t* (98% purity)] were purchased from Matreya, Inc. (Pleasant Gap, PA) and kept at −20 °C. Rhodium, 5% on carbon, was supplied by Strem Chemicals, Inc. (Newburyport, MA), and C_{17} methyl ester, 2-amino-2-methyl-1-propanol (95%), and pancreatic lipase [triacylglycerol (TAG) lipase; EC 3.1.1.3] were purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO). Silica TLC plate F254, 25 cm × 25 cm, was purchased from Merck KGaA (Darmstadt, Germany). Sodium methoxide, ethylene

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glycol, acetonitrile, and hexanes were supplied by Fisher Co. (Fair Lawn, NJ). All chemicals and solvents were reagent grade.

Experimental Design. A central composite rotatable design (*17*) with three variables at five levels, namely, reaction temperature (146, 160, 180, 200, and 214 °C), stirring speed using a magnetic bar controlled by a hot plate heater (100, 200, 300, 400, and 500 rpm), and reaction time (0, 49, 120, 191, and 240 min), was used to determine the maximum yield of CLA.

Isomerization Conditions. The isomerization was performed by mixing 5 g of soybean oil with 10 g of ethylene glycol in a reaction flask. After the mixtures were heated to the set point temperature, 0.015% of rhodium catalyst was added. After the mixture was reacted for the selected reaction time, the isomerized oil was separated from ethylene glycol and catalyst by centrifuging at 2000*g* for 15 min and filtering through a 0.2 μm filter. The isomerized oil was purged with nitrogen and kept at −20 °C. The isomerized soybean oil was analyzed for iodine value by iodometric titration according to AOCS official method Ca 2a-47 (*18*). Fatty acid profiles and CLA were analyzed by GC and HPLC, respectively. CLA isomers were identified by spiking of three standard CLAs in Ag^+ impregnated HPLC analyses. In addition, molecular ion and characteristic fragmentation patterns of 4,4-dimethyloxazoline (DMOX) CLA derivatives were analyzed by GC-MS and compared with reference literature (*19*, *20*). The positions of the CLA in TAGs were investigated by partially hydrolyzing the CLA−TAG with pancreatic lipase. The TAG position was analyzed and confirmed by using TLC and HPLC techniques.

Preparation of CLAME. Approximately 30 mg of isomerized soybean oil was placed into a 15 mL reaction tube fitted with a Teflon-lined screw cap. Two milliliters of 0.5 M sodium methoxide and 1 mL of internal standard (2.02 mg/mL of C_{17} methyl ester in hexane) were added. The tube was flushed with nitrogen and then heated at 50 °C for 20 min with occasional shaking. After methylation was completed, 10 mL of HPLC water was added. The solution was transferred to a 40 mL centrifuge tube, and 6 mL of hexane was added for CLAME extraction. The solution was centrifuged at 2000*g* at 10 °C for 20 min. The hexane layer was dried over sodium sulfate and analyzed by GC and HPLC.

CLAME Analysis by HPLC. A Perkin-Elmer HPLC equipped with a 20 μL Rheodyne injection loop, a UV detector set at 233 nm, and two ChromSpher 5 lipids analytical (4.6 mm i.d. × 250 mm stainless steel, 5 μm particle size, Varian-Chrompack) silver-impregnated columns with a guard column were used in series. HPLC separation was performed isocratically with the mobile phase of 0.1% acetonitrile in hexane freshly prepared and at the flow rate of 1.0 mL/min.

CLAME and Fatty Acid Methyl Ester (FAME) Analysis by GC. CLAME and FAME were analyzed by GC (8500 GC, Perkin-Elmer, Norwalk, CT) equipped with a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc., Bellefonte, PA). Injector and detector temperatures were 240 °C. The column temperature was kept at 75 °C for 1 min and then increased at 20 °C/min to 185 °C and held at 185 °C for 15 min and then increased at 4 °C/min to 220 °C and held at 220 °C for 45 min.

DMOX Derivatives. CLAME samples were collected from HPLC fractions and hydrolyzed to FFAs by using 10 mL of 0.5 N KOH in methanol at 80 °C for 40 min. After hydrolysis, 10 mL of water was added. The mixture was adjusted to pH 3 with 1 N HCl and salted out with NaCl. The mixture was transferred to a 40 mL centrifuge tube, and then, 10 mL of petroleum ether was added and centrifuged at 2000*g* at 10 °C for 20 min. The petroleum ether layer containing FFAs was dried over sodium sulfate and concentrated under nitrogen gas to 1 mL. The sample was then placed into a screw cap reaction tube and a 3-fold amount of 2-amino-2-methyl-1-propanol (*19*) was added. The tube was purged with nitrogen and then heated at 170 °C for 5 h. At the completion of the reaction, 10 mL of HPLC water was added. The mixture was transferred to a 40 mL centrifuge tube, and then, 10 mL of petroleum ether was added. Two milliliters of saturated NaCl was added to break the emulsion. The mixture was centrifuged at 2000*g* at 10 °C for 20 min. The petroleum ether layer was dried over sodium sulfate and concentrated under nitrogen gas. DMOX derivatives were analyzed by GC-MS.

DMOX Derivatives Analysis by GC-MS. DMOX derivatives were analyzed by GC-MS (GC: Varian, Star 3400DX; MS: Varian, Saturn 2000, Palo Alto, CA) equipped with a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc.). Injector and transfer line temperatures were held at 220 °C. The column temperature was kept at 75 °C for 1 min and then increased at 20 °C/min to 185 °C and held at 185 °C for 15 min and then increased at 4 °C/min to 220 °C and held at 220 °C for 45 min.

Statistical Analysis. Results obtained from the central composite rotatable design were analyzed by using the general linear model (GLM) procedure for regression analysis (*21*). An independent sample *t*-test was conducted to identify differences among means. A $p < 0.05$ was considered statistically significant.

Partial Hydrolysis of TAG for CLA Positional Analysis. Twenty-five milligrams of isomerized soybean oil was hydrolyzed with 6 mg of pancreatic lipase in 2.5 mL of 10 M Tris-HCl, pH 8, 0.25 mL of 2.2% w/v $CaCl_2$, and 0.625 mL of 0.05% w/v sodium-taurocholate at 40 °C for 12 min (*22*). The reaction was stopped by adding 5 mL of ethanol and 5 mL of 6.0 N HCl. The mixture was transferred into a 40 mL centrifuge tube, and 10 mL of diethyl ether was added and centrifuged at 2000*g* at 10 °C for 20 min for extraction. The diethyl ether layer was dried over sodium sulfate and concentrated under nitrogen gas. CLA positions in triacyl-*sn*-glycerol were analyzed by TLC and HPLC.

Triacyl-*sn*-glycerol Positional Analysis by TLC and HPLC. The hydrolysate and standards (tripalmitolein, 1-monolinolein, and linoleic acid) were spotted on a silica gel TLC plate. The developing solvent was hexane/diethyl ether/acetic acid (70:30:1 v/v). To detect the positions of the standards and hydrolysates, the TLC plate was partially covered with a glass plate and exposed to iodine vapors. Unexposed TAG fractions were scrapped off the plates and extracted from silica with diethyl ether. TAG, diacylglycerol (DAG), monoacylglycerol (MAG), and FFAs fractions were methylated by BF_3/methanol (*23*) and analyzed for CLA by HPLC.

RESULTS AND DISCUSSION

CLA Profiles of Rh Catalyst Isomerized Oil. Ag^+ HPLC chromatographic separation of CLAME showed 15 peaks in three groups of CLA isomers: *trans*,*trans*, *cis*,*trans*/*trans*,*cis*, and *cis*,*cis*-18:2. Peak identifications for three CLAs were confirmed to be 9*t*,11*t*-18:2, 9*c*,11*t*-18:2, and 9*c*,11*c*-18:2 by spiking with standard CLAs of 98% purity. Further identification of other CLA peaks was done by analyzing DMOX derivatives from Ag^+ HPLC fractions by GC-MS and using reference literature (*19*, *20*). The DMOX derivative spectra showed molecular ions of *m*/*z* 333, which is a molecular ion of the DMOX derivative of all CLA isomers (*24*). The mass spectra consisted of a series of even mass ions separated by 14 mass units (mu) due to losses of methylene units. The even mass homologous series *m*/*z* 126 + 14 mu is interrupted in the region of the double bonds. The mass spectrum showed a mass difference of 12 mu between *m*/*z* 210/222 and *m*/*z* 236/248, *m*/*z* 196/208, and *m*/*z* 222/234 according to conjugated double bonds in positions 10,12 and 9,11, respectively (*19*, *24*, *25*).

From the results of CLA standard spiking, MS of DMOX derivatives, and information from references, it can be concluded that the *trans*,*trans*-18:2 was the first eluted group followed by *cis*,*trans*/*trans*,*cis*-18:2 and then *cis*,*cis*-18:2. The *trans*,*trans* isomers were separated into six peaks identified as (1) 12*t*,14*t*, (2) 11*t*,13*t*, (3) 10*t*,12*t*, (4) 9*t*,11*t*, (5) 8*t*,10*t*, and (6) 7*t*,9*t*. The *cis*,*trans*/*trans*,*cis* isomer group contained five isomers and was the major group of CLA in the isomerized oil; (7) 12*c*,14*t*/12*t*,-14*c*, (8) 11*c*,13*t*/11*t*,13*c*, (9) 10*c*,12*t*/10*t*,12*c*, (10) 9*c*,11*t*/9*t*,-11*c*, and (11) 8*c*,10*t*/8*t*,10*c* as shown in **Figure 1**. The last CLA group on Ag^+ HPLC chromatogram included the *cis*,*cis* isomers. They separated into four peaks as (12) 11*c*,13*c*, (13) 10*c*,12*c*, (14) 9*c*,11*c*, and (15) 8*c*,10*c*.

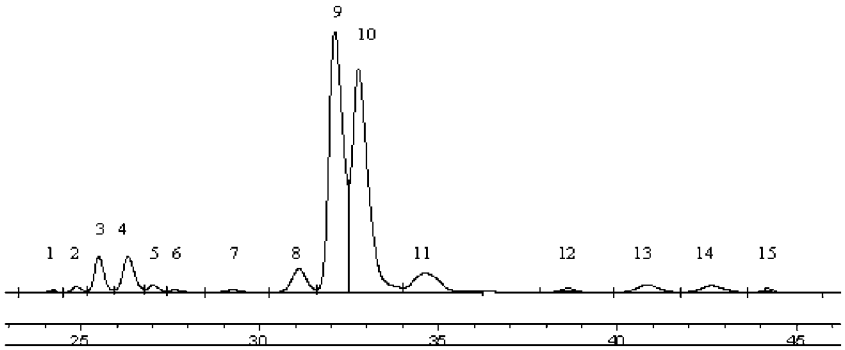

Figure 1. Ag^+ HPLC chromatogram of isomerized soybean oil. The individual isomers are (1) 12*t*,14*t*; (2) 11*t*,13*t*; (3) 10*t*,12*t*; (4) 9*t*,11*t*; (5) 8*t*,10*t*; (6) 7*t*,9*t*; (7) 12*c*,14*t*/12*t*,14*c*; (8) 11*c*,13*t*/11*t*,13*c*; (9) 10*c*,12*t*/10*t*,12*c*; (10) 9*c*,11*t*/9*t*,11*c*; (11) 8*c*,10*t*/8*t*,10*c*; (12) 11*c*,13*c*; (13) 10*c*,12*c*; (14) 9*c*,11*c*; and (15) 8*c*,10*c*.

Table 1. Effects of Isomerization Conditions on Total CLA Contents and Iodine Values of Isomerized Soybean Oil

treatment	temp (°C)	speed (rpm)	time (min)	total CLA (mg/g of oil)	iodine value
1	160	200	49	112.7 ± 7.5	120 ± 1
2	200	200	49	202.4 ± 22.1	115 ± 1
3	160	400	49	129.8 ± 13.4	118 ± 1
4	200	400	49	181.9 ± 13.9	116 ± 1
5	160	200	191	86.4 ± 7.9	118 ± 1
6	200	200	191	117.1 ± 11.3	116 ± 1
7	160	400	191	86.3 ± 9.4	116 ± 1
8	200	400	191	121.7 ± 12.9	113 ± 1
9	146	300	120	88.0 ± 11.5	122 ± 1
10	214	300	120	63.2 ± 10.9	111 ± 1
11	180	100	120	111.1 ± 3.2	119 ± 1
12	180	500	120	117.5 ± 14.5	118 ± 1
13	180	300	0	2.3 ± 0.3	136 ± 1
14	180	300	240	105.7 ± 12.8	111 ± 0
15	180	300	120	134.3 ± 8.3	116 ± 0

Effects of Isomerization Conditions on the CLA Contents. Preliminary analysis showed that the rhodium catalyst on carbon was more effective for isomerization than palladium and platinum. Using hydrogen gas as the hydrogen source favored hydrogenation rather than isomerization, whereas using ethylene glycol as the hydrogen source was optimal for the conversion of linoleic acid to CLA, which might involve the concentration of chemisorbed hydrogen (*26*). Furthermore, we found that isomerization did not occur at low temperature, and long reaction times induced hydrogenation rather than conjugation. Thus, the study design included high reaction temperatures for less than 4 h. CLA concentrations were determined by integration of GC peak areas using C_{17} fatty acid as an internal standard. The original soybean oil contained palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid contents of 122.80, 42.60, 223.80, 550.30, and 67.70 mg/g oil, respectively, and a total CLA concentration of 0.63 mg/g oil, which increased to 2.33 mg/g oil (treatment 13) by simply being heated from room temperature to 180 °C (**Table 1**).

As it can be seen from **Table 1**, the formation of CLA during isomerization was greatly dependent on reaction temperature and time. Increasing the reaction temperature from 146 (treatment 9) to 180 °C (treatment 15) resulted in an increase in total CLA contents (**Figure 2A**). This was due to the isomerization reaction of linoleic acid, which decreased as reaction temperatures increased (**Figure 2B**). Another source for CLA might have been linolenic acid as it decreased from 67.70 mg/g of oil in the starting soybean oil to 12.10 mg/g of oil in treatment 2.

The maximum yield of total CLA was 202.42 mg/g oil obtained at 200 °C with a stirring speed of 200 rpm for 49 min (treatment 2) (**Table 1**). At this temperature, Jung et al. (*16*) also found high CLA contents in partially hydrogenated soybean oil using a nickel catalyst. They reported a total CLA content of 162.82 mg/g oil at a hydrogenation temperature of 210 °C; however, the *trans*,*trans* isomers were also very high.

When the temperature increased to 214 °C (treatment 10), the total CLA significantly ($p < 0.05$) decreased due to hydrogenation, which was determined by a decrease in iodine value from 115.53 in treatment 2 to 111.26 in treatment 10 ($p < 0.05$). The supporting information is shown by an increase in C18:1 and C18:0 as a result of loss of double bonds from C18:2 and C18:3 (**Figure 2C**,**D**). In addition, when the reaction time was long (4 h; treatment 14), the total CLA decreased to 105.73 mg/g oil. It was due to hydrogenation being favored over isomerization as indicated by an increase of monounsaturated and saturated fatty acids (**Figure 2C**,**D**) and a decrease in iodine value to 111.17. Jung et al. (*16*) also found that the hydrogenation of soybean oil significantly increased when a nickel catalyst was used in the reaction for 3−4 h.

The proportions of individual CLA isomers affected by the isomerization conditions are shown in **Table 2**. Only reaction temperatures and times affected the alteration of CLA configuration. Increasing the temperature to 180 °C resulted in an increase in *cis*,*trans*/*trans*,*cis* isomers. A further increase in the reaction temperatures caused a decrease in *cis*,*trans*/*trans*,*cis*-CLA, which resulted from bond shifting of *cis*,*trans*/*trans*,*cis*- to *trans*,*trans*-CLA. The reaction time also affected *cis*,*trans*/*trans*,*cis*-CLA proportions. A long reaction time (4 h) caused a decrease in *cis*,*trans*/*trans*,*cis* isomer but *trans*,*trans*-CLA increased.

The maximum CLA yield was obtained from treatment 2. This isomerization condition provided 20.56, 72.46, and 7.00% of *trans*/*trans*-, *cis*,*trans*/*trans*,*cis*-, and *cis*,*cis*-CLA, respectively. The amounts of isomers 10*c*,12*t*/10*t*,12*c* (34.84%) and 9*c*,11*t*/9*t*,11*c* (28.78%) indicated that the double bond at Δ9, which was closer to the carboxyl group, had a greater absorption of Rh and was more reactive than the double bond at Δ12, which is closer to the methyl end of the carbon chain. Therefore, it might be concluded that the isomerization condition at 200 °C, 200 rpm for 49 min, provided CLA contents high in *cis*,*trans*/*tran*,*cis* isomers, of which 9*c*,11*t* and 10*t*,12*c* have been reported to have anticarcinogen and body fat reduction properties. In addition, this isomerization condition exhibited rather low concentrations of *trans*,*trans*- and *cis*,*cis*-CLA isomers. As compared to a nickel catalyst (*16*), rhodium provided much higher proportions of *cis*,*trans*/*tran*,*cis*- and lower amounts of *trans*,*trans*-CLA. However, alkaline isomerization (*27*) has a greater activity and selectivity than rhodium because it provided

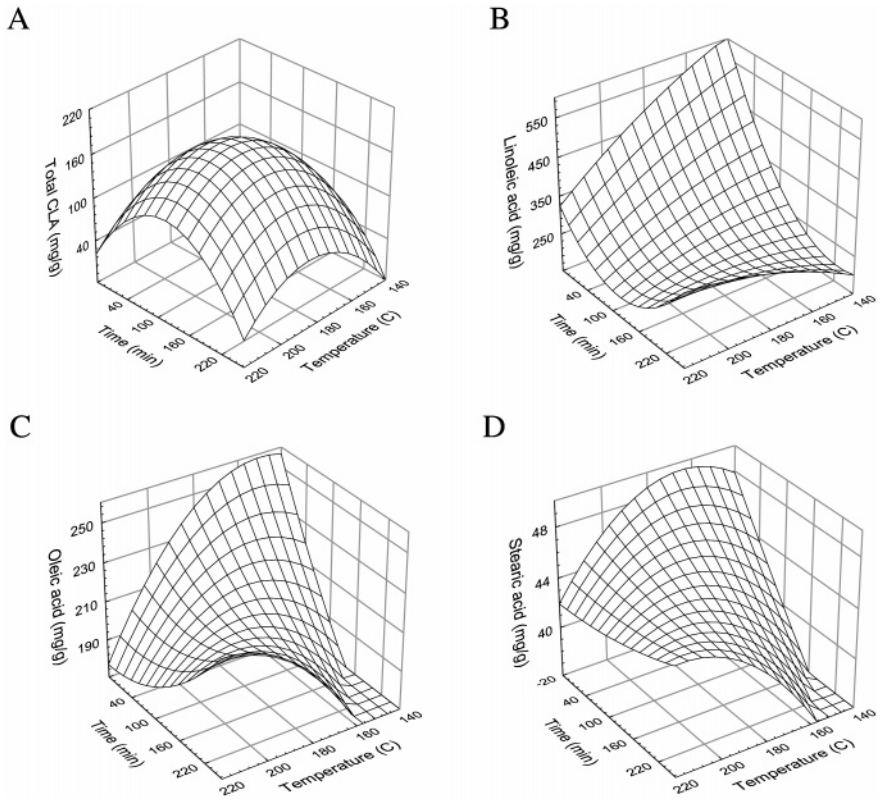

Figure 2. Response surface graphs showing the effect of temperature and isomerization time at a stirring speed of 200 rpm on total CLA (**A**), linoleic acid (**B**), oleic acid (**C**), and stearic acid (**D**).

Table 2. Distribution of Individual CLA Isomers (% of Total CLAs) Affected by Isomerization Conditions

	treatments														
CLA isomers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12*t*,14*t*	0.16	0.41	0.35	0.36	0.48	0.33	0.29	0.42	0.13	1.06	0.34	0.17	0.00	0.34	0.29
11*t*,13*t*	2.37	2.61	1.68	2.22	1.70	2.04	1.94	2.41	1.82	3.63	2.06	2.19	0.00	2.20	2.38
10*t*,12*t*	6.28	5.76	6.63	6.95	7.09	7.06	7.43	7.75	6.75	9.81	7.06	6.85	8.86	7.92	7.27
9*t*,11*t*	6.27	7.65	6.61	6.87	7.08	6.97	7.40	7.69	6.76	10.14	7.06	6.82	12.15	7.91	7.23
8*t*,10*t*	2.62	3.98	2.96	3.35	2.78	2.94	2.76	3.46	2.35	5.28	3.14	2.88	4.81	3.47	3.13
7*t*,9*t*	0.17	0.15	0.36	0.46	0.08	0.29	0.08	0.19	0.03	0.22	0.30	0.11	0.90	0.11	0.15
12*c*,14*t*/12*t*,14*c*	0.97	0.13	0.94	0.62	0.28	0.67	0.21	0.37	0.24	1.64	0.48	0.26	0.94	0.12	0.29
11*c*,13*t*/11*t*,13*c*	1.19	4.40	1.42	4.30	2.05	9.60	2.04	8.25	1.18	13.16	4.20	3.80	4.78	5.61	3.67
10*c*,12*t*/10*t*,12*c*	37.97	34.84	38.89	34.79	38.14	29.08	38.16	29.35	40.84	17.25	34.83	36.20	17.82	32.56	36.20
9*c*,11*t*/9*t*,11*c*	32.08	28.78	31.76	28.75	30.96	23.97	31.71	24.76	33.22	16.22	29.82	30.47	20.92	27.05	30.20
8*c*,10*t*/8*t*,10*c*	1.56	4.31	1.47	4.26	2.38	9.50	2.32	8.12	1.62	12.65	4.34	3.71	2.46	5.83	3.66
11*c*,13*c*	0.68	0.41	0.30	0.49	0.27	0.47	0.21	0.50	0.17	0.00	0.46	0.34	0.79	0.50	0.30
10*c*,12*c*	3.76	3.08	2.99	3.04	3.32	3.20	2.74	3.31	2.46	2.16	2.80	3.11	15.45	3.04	2.54
9*c*,11*c*	3.39	3.01	2.98	2.84	3.09	3.27	2.41	3.02	2.11	3.47	2.61	2.71	7.16	3.08	2.33
8*c*,10*c*	0.52	0.50	0.67	0.70	0.31	0.61	0.32	0.42	0.32	3.31	0.50	0.37	2.96	0.26	0.36
total *trans*,*trans*	17.87	20.56	18.59	20.21	19.21	19.63	19.90	21.92	17.84	30.14	19.96	19.02	26.72	21.95	20.45
total *cis*,*trans*/*trans*,*cis*	73.77	72.46	74.48	72.72	73.81	72.82	74.44	70.85	77.1	60.92	73.67	74.44	46.92	71.17	74.02
total *cis*,*cis*	8.35	7.00	6.94	7.07	6.99	7.55	5.68	7.25	5.06	8.94	6.37	6.53	26.36	6.88	5.53

higher proportions of *cis*,*trans*/*tran*,*cis*- and lower amounts of *trans*,*trans*-CLA. The catalytic pathway for double bond migration of linoleic acid by heterogeneous catalysts was clearly described by Bernas et al. (*28*).

Analysis of CLA Position. Investigation of acyl selection during isomerization was done by partially hydrolyzing the TAG structure of the isomerized oil using enzyme pancreatic lipase. This enzyme has the ability to cleave the fatty acids at *sn*-1 and *sn*-3 positions. The four fractions of FFAs, MAG, DAG, and TAG shown on the TLC plate were reported by Valle et al. (*22*) to be FFAs and *sn*-2 MAG bands representing the position of *sn*-1, -3, and -2 of TAG, respectively. Individual fractions were methylated by BF_3/methanol and analyzed with HPLC to estimate the total CLA content and for monitoring the effect of Rh heterogeneous catalyst on the conversion of linoleic acid to CLA at the various *sn*-TAG positions. The HPLC chromatogram showed each fraction of hydrolysate contained high amounts of CLA. However, the intensity of the FFA fraction was almost double that of the MAG fraction, which indicates that fatty acids in both, *sn*-1 and -3 positions can be isomerized to CLA. However, the results do not reveal the % conversion differences between *sn*-1 and -3, but according to Valle et al. (*22*), it could be possible that linoleic acid at any position in triacyl-*sn*-glycerol could be isomerized to CLA.

In conclusion, conjugation of unsaturated fatty acids in vegetable oil can be done by using rhodium heterogeneous catalyst at high temperatures. Isomerization conditions set up in this experiment definitely provided high CLA−TAG contents

in soybean oil up to 202.42 mg/g, which contained 63.62% of beneficial 9*c*,11*t* and 10*t*,12*c* isomers. Only 15 g of isomerized oil would have a CLA content that is high enough to match the daily recommended (*20*) dose of 3 g/day.

Supporting Information Available: Statistical analysis of the GLM procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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