Effects of Temperature and Agitation Rate on the Formation of Conjugated Linoleic Acids in Soybean Oil during Hydrogenation Process

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The effects of hydrogen temperature and agitation rate on the formation of total conjugated linoleic acids (CLA) and CLA isomers were studied during hydrogenation with a selective Ni catalyst. The CLA isomers were identified by using a 100-m cyano-capillary column gas chromatograph and a silver ion-impregnated HPLC. Reaction temperature and agitation rate greatly affected the quantities of total CLA and individual CLA isomers, and the time to reach the maximum quantity of CLA in the partially hydrogenated soybean oil. As the hydrogenation temperature increased, the maximum quantity of CLA in soybean oil increased, but the time to reach the maximum CLA content decreased. By increasing the hydrogenation temperature from 170 to 210 °C, the quantity of CLA obtained was about 2.6 times higher. As the agitation rate decreased, the CLA formation in soybean oil increased, and the time to reach the maximum CLA content also increased. The maximum CLA contents in soybean oil obtained during hydrogenation at 210 °C with agitation rates of 300, 500, and 700 rpm were 162.82, 108.62, and 66.15 mg total CLA/g oil, respectively. The present data showed that it is possible to produce high-CLA-content soybean oil without major modification of fatty acid composition by short-time (10 min) selective hydrogenation under high temperature and low agitation rate conditions.

Keywords: *Conjugated linoleic acids (CLA); soybean oil; hydrogenation; temperature; hydrogen pressure*

INTRODUCTION

Conjugated linoleic acids (CLA) are a group of naturally occurring isomers of linoleic acid containing a conjugated double bond system. CLA have been recognized for their ability to prevent or cure cancer (*1*−*7*), atherosclerosis (*8, 9*), and Type II diabetes (NIDDM) (*10*). It also has been reported that CLA are involved in the regulation of cytokines production, resulting in muscle and bone strengthening activity (*9, 11, 12*). Fat partitioning activity of CLA also has been reported, resulting in fat reduction in pigs and humans (*9, 13, 14*).

CLA are abundant in dairy products and meats from ruminant animals (*15*−*20*). Ha et al. (*15*) originally reported high levels of CLA in cheeses and milk. Chin et al. (*17*) reported that dairy products (milk, butter, cheese, and yogurt) and meats from ruminant animals contained large quantities of CLA (ca. 3−8 mg total CLA/g fat). The authors also reported that vegetable oils contained very low levels of CLA, ranging from 0.1 mg CLA/g oil (coconut oil) to 0.7 mg CLA/g oil (safflower oil).

Mossoba et al. (*21*) found that cis−trans and trans−cis isomers of linoleic acid were present in hydrogenated soybean oil and margarine by means of capillary gas chromatography−matrix isolation−Fourier transform infrared spectroscopy. Banni et al. (*22*) carried out a series of HPLC/UV/MS analyses to characterize the fatty acids with conjugated dienes in a partially hydrogenated oil (mixture of partially hydrogenated soybean oil and palm oil). The authors concluded that the partially hydrogenated oil contained conjugated linoleic acid isomers. Banni et al. (*23*) reported that a partially hydrogenated vegetable oil contained 4.24 mg total CLA/g oil. Jung and Ha (*24)* studied conjugated linoleic acids formation during selective and nonselective hydrogenation processes, and they found that the large quantity of linoleic acids in soybean oil was formed during selective hydrogenation processes. The authors reported that the maximum CLA content produced in soybean oil was 98.27 mg, which was among the highest ever reported in foods. The hydrogenation is performed under various conditions to produce the hydrogenated oil with different physical and chemical properties. It has been generally known that the reaction conditions, especially hydrogenation temperature and agitation rate, greatly affect the types of fatty acid isomers formed in vegetable oils. However, the effects of different hydrogenation conditions such as hydrogenation temperature and agitation rate on the formation of conjugated linoleic acids in vegetable oils have never been previously reported.

The objectives of this research were to determine the effects of different hydrogenation temperature and

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agitation rate on the quantity of total CLA and CLA isomers in soybean oil during hydrogenation processes.

MATERIALS AND METHODS

Materials. Authentic CLA methylesters, stearic acid methylester, oleic acid methylester, linoleic acid methyl ester, linolenic acid methylester, arachidic acid methylester, behenic acid methylester, and heptadecanoic acid methylester were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC-grade hexane and acetonitrile were purchased from Mallinckrodt Specialty Chem. Co. (Paris, KY). Sodium methoxide in methanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Butter (Seoul Fresh Butter, Seoul Milk Ltd., Seoul, Korea) and cream cheese (Soft Philadelphia Cream Cheese, Kraft Foods) were purchased from a local grocery. RBD (refined, bleached, and deodorized) soybean oil without any additive was obtained from Samlyp Oil and Fat Ltd. (Seoul, Korea). The peroxide value of the soybean oil was 0 meq/kg oil. The fatty acid composition of soybean oil was 10.53% palmitic acid, 4.29% stearic acid, 22.19% oleic acid, 1.33% *cis*-18:1, 0.63% unconjugated linoleic acid isomers, 52.99% linoleic acid, 0.37% arachidic acid, 1.11% linolenic acid isomers, 6.17% linolenic acids, and 0.38% behenic acid

Hydrogenation Conditions for the Preparation of Partially Hydrogenated Soybean Oils. The hydrogenation was performed with a 1-L capacity hydrogenation reactor (Next Instrument, Hwa-sung, Korea) equipped with temperature and agitation rate controller. The hydrogenation was carried out with 0.1% of a commercially available selective Ni catalyst (SP-7, Engelhard) under various temperatures (170 ± 2, 190 ± 2, or 210 ± 2 °C) and agitation rates (300, 500, and 700 rpm). The hydrogen pressure used was 0.5 kg/cm^2 during the hydrogenation process. The oil samples (10 g each) were collected at the predetermined interval during hydrogenation.

Preparation of Fatty Acid Methyl Esters. For extracting fats from butter and cheese, diethyl either (300 mL) was added to butter or cheese, and was stirred with a glass rod. The diethyl ether fraction was transferred into a 2000-mL capacity separatory funnel and then 500 mL of saturated NaCl solution was added to the funnel. After being shaken for a sufficient time, the separatory funnel stood until two distinct layers were clearly separated. The bottom water layer was discarded to remove the water soluble components in the sample, and this washing step was repeated two more times. The diethyl ether was evaporated from the collected diethyl ether layer by using a rotatory vacuum evaporator at 35 °C. The obtained butter and cheese oils were used for the methylesterification. Soybean oil samples were methylesterified without any pretreatment. Methylesterification of the hydrogenated soybean oils, butter, and cheese fat was carried out with sodium methoxide (0.25 N) in methanol at 70 °C for 20 min. The fatty acids of methylester (FAME) were extracted with 2,2,4-trimethylpentane containing internal standard (heptadecanoic acid).

Silver Ion High Performance Liquid Chromatography. Silver ion high-performance liquid chromatographic separation of conjugated linoleic acid methyl esters was carried out using an HPLC (Shimazu, Tokyo, Japan) equipped with a 20-μL injection loop (Waters) and UV detector operated at 233 nm (*25*). Three ChromSpher 5 Lipids analytical silver-impregnated columns (each 4.6 mm i.d. x 250 mm stainless steel; 5 μm particle size; Chrompack, Bridgewater, NJ) were used in series. The mobile phase was 0.1% acetonitrile in hexane and operated isocratically at a flow rate of 1.0 mL/min.

Gas Chromatography. Isolation of conjugated linoleic acids was carried out by gas chromatography. FAME (fatty acid methylester) samples of 2−6 μL each were injected into a gas chromatograph equipped with a flame ionization detector. The column used was a highly polar, fused-silica capillary column (cyanopropyl siloxane phase, SP3480 100 m × 0.25 mm, 0.25 μm thickness, Supelco Inc., Bellefonte, PA). The 100:1 split injection was used for sample injection. Helium was used as a carrier gas with a head pressure of 300 kPa. Temperatures of injector and detector were 250 and 250 °C, respectively. Initial oven temperature was 170 °C held for 1 min and then increased 0.8 °C/min to 200 °C. Analytical results for FAME of CLA isomers were expressed as follows: weight of CLA = peak area of CLA/peak area of internal standard and normalized to weight of internal standard.

For the partially hydrogenated soybean oils and butter, the CLA contents were calculated as mg/g of oil by using the formula

$$\text{CLA (mg/g)} = (A_x)(W_{IS})(CF_x)/(A_{IS})(W_s)(1.04)$$

where A_x = peak area of CLA, A_{IS} = peak area of internal standard, CF_x = theoretical correction factor for CLA calculated based on internal standard, W_{IS} = weight of internal standard added to the sample (in mg), W_s = sample weight (in g). The conversion factor 1.04 was adopted from the previous work (*26*) to express the results as mg fatty acid/g fat rather than as methylesters. Because the conjugated linoleic acid isomers have identical active carbon numbers and unsaturation, all the conjugated linoleic acid isomers have the same theoretical detector response (*27*). Thus, the same correction factor for different CLA isomers was used (*26*). The theoretical correction factor (CF_x) was calculated by using the theoretical detector response of internal standard (hexadecanoic acid) compared to that of conjugated linoleic acids (*27*). The relative theoretical detector responses for the same weight of methyl esters of C17:0, C18:0, and C18:2 are 0.991:1:1.013. Thus, the calculated theoretical correction factor (CF_x) is 0.991/1.013 = 0.978.

Iodine Value. Iodine values of the partially hydrogenated soybean oils were determined by AOCS official method Cd 1c-85 (*28*).

RESULTS AND DISCUSSION

CLA Identification. Soybean oil was hydrogenated with a selective catalyst at different temperatures and agitation rates. Figure 1 shows the partial Ag^+−HPLC chromatograms of authentic conjugated linoleic acids, cheese fatty acid methylesters, and hydrogenated soybean oil methylesters. The hydrogenated soybean oil was obtained after 80 min of hydrogenation under the conditions of 210 °C and 500 rpm agitation rate. The elution patterns of chromatographic peaks for the authentic CLA methylesters and cheese fatty acid methylesters were exactly the same as those reported previously (*29*). The individual Ag^+−HPLC peak of the hydrogenated soybean oil was identified by comparison with those of authentic CLA standard and cheese CLA which were previously reported in the literature (*25, 29*). Two peaks (g and h) could not be identified by comparing the retention time with authentic CLA standard and cheese CLA, because these peaks in authentic CLA and cheese were not identified in the previous reports. But, on the basis of expected elution order as previously reported, we assumed that these peaks were 12c,14t and 12t,14c isomers. To confirm the peak identification, two fractions of these Ag^+−HPLC chromatographic peaks (g and h) were collected and injected into a gas chromatograph. Both fractions of peak g and h showed a single gas chromatographic peak. The fraction of peak h was eluted before the fraction of peak g in the gas chromatography. Peak g was coeluted with 10c,12c and peak h was coeluted with 10t,12c. On the basis of this analysis by gas chromatography and Ag^+−HPLC, and expected elution order in both Ag^+−HPLC and gas chromatography, peaks g and h were tentatively identified as 12t,14c and 12c,14t CLA isomers, respectively. The collected fraction of peak k showed two peaks in the gas chromatography. The peak elution order in the gas chromatography showed that peak k is a mixture

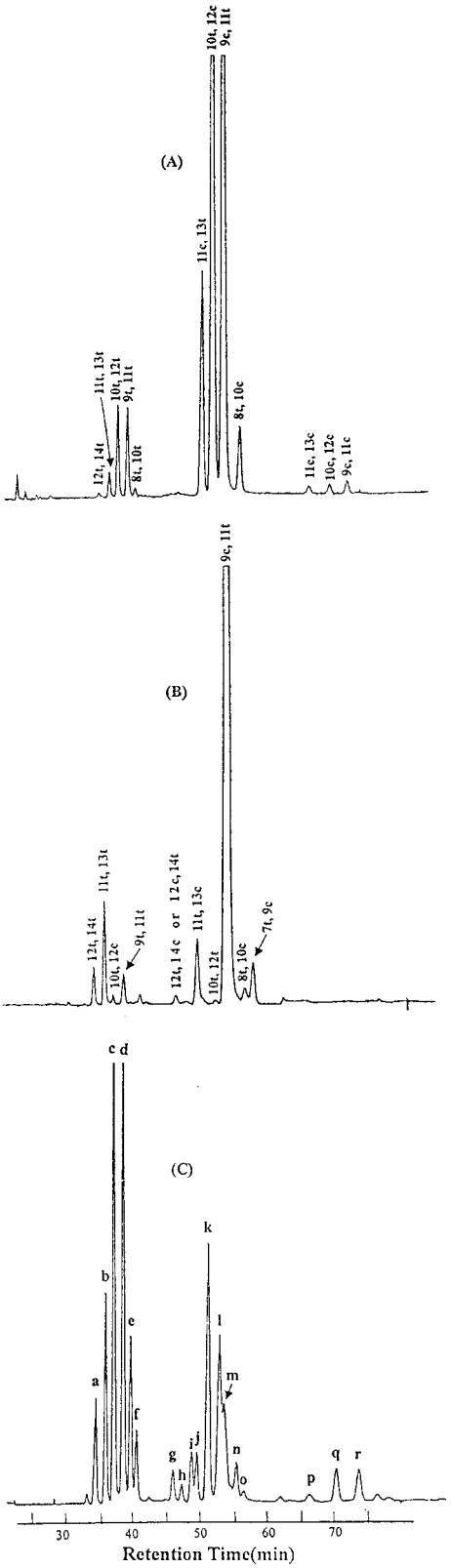

Figure 1. Partial Ag^+−HPLC chromatograms for (A) authentic CLA methylesters, (B) cheese fatty acid methylesters, and (C) partially hydrogenated soybean oil fatty acid methylesters. Peaks a−r represent 12t,14t (peak a); 11t,13t (peak b); 10t,-12t (peak c); 9t,11t (peak d); 8t,10t (peak e); 7t,9t (peak f); 12t,-14c (peak g); 12c,14t (peak h); 11t,13c (peak i); 11c,13t (peak j); 10t,12c/10c,12t (peak k); 9c,11t (peak l); 9t,11c (peak m); 8t,10c (peak n); 7t, 9c (peak o); 11c,13c (peak p); 10c,12c (peak q); and 9c,11c CLA isomers (peak r), respectively.

of 10t,12c and 10c,12t CLA isomers. The isomers of Ag^+−HPLC peaks of the hydrogenated soybean oil were identified as 12t,14t (peak a), 11t,13t (peak b), 10t,12t (peak c), 9t,11t (peak d), 8t,10t (peak e), 7t,9t (peak f), 12t,14c (peak g), 12c,14t (peak h), 11t,13c(peak i), 11c,13t (peak j), 10t,12c/10c,12t (peak k), 9c,11t (peak l), 9t,11c (peak m), 8t,10c (peak n), 7t, 9c (peak o), 11c,13c (peak p), 10c,12c (peak q), and 9c,11c CLA isomers (peak r).

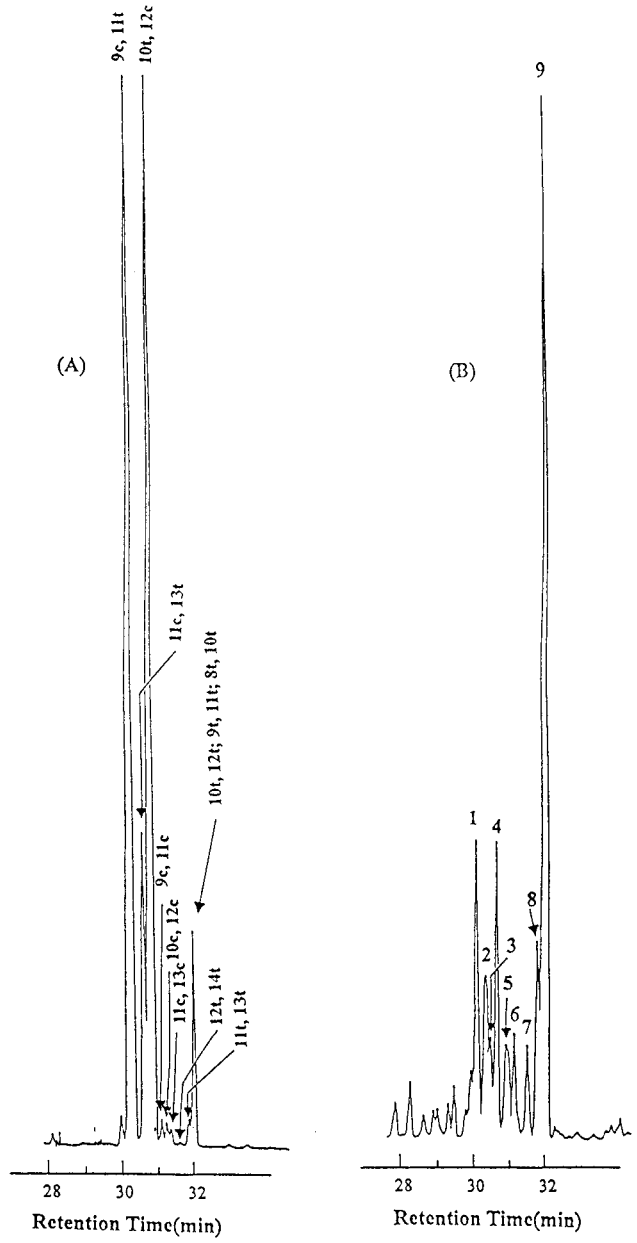

Figure 2. Gas chromatograms of (A) authentic CLA methylesters and (B) partially hydrogenated soybean oil fatty acid methylesters. Peaks 1−9 represent 7t,9c/9c,11t/8t,10c (peak 1); 10c,12t (peak 2); 9t,11c/11c,13t (peak 3); 12c,14t/10t,12c (peak 4); 11t,13c/9c,11c (peak 5); 12t,14c/10c,12c/11c,13c (peak 6); 12t,14t (peak 7); 11t,13t (peak 8); and 10t,12t/9t,11t/8t,-10t/7t,9t CLA isomers (peak 9), respectively.

Figure 2 shows gas chromatograms of hydrogenated soybean oil and authentic CLA isomers. To identify the individual gas chromatographic peak of authentic CLA and hydrogenated soybean oil, each fraction of Ag^+−HPLC chromatographic peak of authentic CLA and hydrogenated soybean oil was collected by repeated Ag^+−HPLC runs, and the collected fractions were injected into the gas chromatograph. Each peak of gas chromatography was identified as 7t,9c/9c,11t/8t,10c

Table 1. Iodine Values of the Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under Different Reaction Conditions

170 °C; 500 rpm		190 °C; 500 rpm		210 °C; 500 rpm		210 °C; 700 rpm		210 °C; 300 rpm	
HT[a] (min)	IV[b]	HT[a] (min)	IV	HT[a] (min)	IV	HT[a] (min)	IV	HT[a] (min)	IV
0	132.1	0	132.1	0	132.1	0	132.1	0	132.1
20	128.2	20	126.6	10	127.3	10	125.9	10	129.3
40	124.7	40	121.9	20	124.2	20	120.1	20	127.9
60	121.7	60	118.2	30	120.9	30	114.0	40	125.9
80	118.3	80	113.4	40	117.8	40	109.5	60	122.7
100	115.9	100	110.4	50	115.4	50	103.7	80	119.5
120	113.0	120	107.3	60	111.9	60	98.5	100	118.6
140	110.1	140	104.0	70	108.4	70	94.5	120	116.0
160	108.0	160	100.8	80	107.4	80	90.1	140	113.6
180	105.0	180	96.5	90	104.5	90	85.3	160	112.5
200	102.9	200	92.8	100	102.8			180	110.3
220	100.3	220	89.7	110	99.6			200	108.3
240	97.9	240	86.7	120	96.0			220	104.9
260	95.8	260	83.1	130	93.7			240	104.1
280	92.4			140	92.8			260	102.0
300	91.3			160	87.2			280	100.5
320	89.6							300	97.4
								320	95.3

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [b] IV, iodine value of hydrogenated soybean oil obtained after certain hydrogenation time.

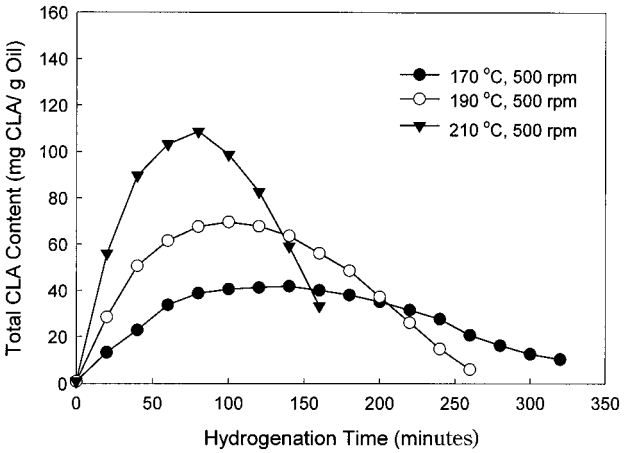

Figure 3. Effects of reaction temperature on the total CLA contents in soybean oil during the hydrogenation process.

(peak 1), 10c,12t (peak 2), 9t,11c/11c,13t (peak 3), 12c,-14t/10t,12c (peak 4), 11t,13c/9c,11c (peak 5), 12t,14c/10c,12c/11c,13c (peak 6), 12t,14t (peak 7), 11t,13t (peak 8), and 10t,12t/9t,11t/8t,10t/7t,9t-CLA isomers (peak 9), respectively. The gas chromatographic elution order of the CLA isomers was exactly the same as the expected one as previously suggested by Sehat et al. (*25*, *29*), indicating the correct identification of individual CLA isomers in hydrogenated soybean oil.

Effects of Reaction Temperature on the CLA Contents in Soybean Oil during Hydrogenation Process. The changes in iodine values of the soybean oil during hydrogenation under different reaction conditions are shown in Table 1. Figure 3 shows the effects of reaction temperature on the total CLA contents in soybean oil during hydrogenation processes. Tables 2, 3, and 4 show the contents of individual CLA isomers in soybean oil during hydrogenation at 170, 190, and 210 °C, respectively. The hydrogenation was carried out under the agitation rate of 500 rpm and 0.5 kg/cm^2 hydrogen pressure. Soybean oil before hydrogenation contained only 0.86 mg trans, trans CLA isomers (peak 9)/g oil. The CLA content increased initially with hydrogenation time, and after reaching maximum, the content decreased. Reaction temperature during hydrogenation greatly affected the quantities of total CLA and

Table 2. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under the Conditions of 170 °C and 500 rpm

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)								
(min)	1	2 + 3	4	5	6	7	8	9	total
0	t[c]	ND[d]	ND	ND	ND	ND	ND	0.86	0.86
20	3.45	0.82	3.36	0.73	1.00	0.18	0.27	3.45	13.27
40	4.91	1.64	5.00	1.27	1.09	0.09	1.09	7.72	22.81
60	6.63	2.91	6.00	1.91	1.45	0.73	1.73	12.29	33.65
80	7.09	3.63	5.91	2.09	1.64	0.91	2.18	15.36	38.80
100	6.91	3.82	5.27	2.27	1.54	0.91	2.45	17.35	40.52
120	6.36	4.09	5.18	2.00	1.54	1.00	2.63	18.44	41.25
140	6.36	4.36	4.72	2.00	1.45	1.09	2.63	19.17	41.80
160	5.91	4.09	4.27	1.91	1.36	1.09	2.63	18.81	40.07
180	5.45	3.91	3.91	1.73	1.27	1.18	2.63	17.99	38.07
200	4.91	3.73	3.45	1.64	1.09	0.91	2.27	17.08	35.07
220	4.36	3.45	3.00	1.18	1.18	0.82	2.09	15.45	31.53
240	3.82	2.91	2.54	1.00	1.18	0.82	1.54	13.81	27.62
260	2.09	2.00	1.91	0.82	0.64	0.09	1.45	11.72	20.72
280	1.27	1.73	1.54	0.73	0.45	0.09	1.36	9.18	16.35
300	1.36	1.36	1.00	0.09	0.09	0.09	1.09	7.54	12.63
320	1.00	1.00	0.64	0.45	0.36	0.27	0.91	5.82	10.45

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [b] Identity of each isomer as shown in Figure 2. [c] t, trace amount (less than 0.1 mg/g oil). [d] ND, not detected.

CLA isomers, and the time to reach the maximum quantity of CLA in the partially hydrogenated soybean oil. As the hydrogenation temperature increased, the maximum quantity of CLA in soybean oil increased. The maximum CLA contents obtained in soybean oil during hydrogenation at 170, 190, and 210 °C were 41.90, 69.60, and 108.67 mg CLA/g oil, respectively (Figure 3). That is, by increasing the hydrogenation temperatures from 170 to 210 °C, the quantity of CLA obtained was about 2.6 times higher.

As the hydrogenation temperature increased, the time required to reach the maximum CLA content decreased (Figure 3). Maximum CLA content in soybean oil was obtained after 120, 100, and 80 min of hydrogenation at the temperatures of 170, 190 and 210 °C, respectively. To check the accuracy of the CLA contents in the partially hydrogenated soybean oils, we also analyzed the CLA content in butter. Our analytical result showed that the butter contained 4.58 mg total CLA/g oil(data

Table 3. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under the Condition of 190 °C and 500 rpm

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil)								
	1	2 + 3	4	5	6	7	8	9	total
0	t[c]	ND[d]	ND	ND	ND	ND	ND	0.86	0.86
20	6.27	1.91	6.54	1.73	1.45	0.36	1.18	9.00	28.44
40	9.81	4.63	8.54	2.82	2.18	1.09	2.82	18.72	50.61
60	10.72	5.91	8.54	3.54	2.45	1.45	3.63	25.26	61.51
80	10.81	7.00	8.18	3.63	2.63	1.82	4.54	28.98	67.60
100	10.63	7.18	7.63	3.82	2.63	2.00	4.82	30.89	69.60
120	9.99	6.27	7.54	3.54	2.63	2.09	4.72	30.98	67.78
140	9.36	6.00	6.27	3.63	2.18	2.09	4.54	29.53	63.60
160	7.81	5.27	5.54	3.12	2.09	1.91	4.18	26.26	56.18
180	6.72	4.45	4.72	2.63	1.82	1.64	4.18	22.44	48.61
200	5.63	4.18	2.82	2.09	1.45	1.18	2.82	17.08	37.25
220	3.54	2.36	2.36	1.45	1.00	0.91	2.45	11.99	26.08
240	1.91	1.45	1.18	0.91	0.55	0.55	1.27	7.09	14.90
260	0.73	0.45	0.36	0.27	0.18	0.18	0.64	3.27	6.09

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time. [b] Identity of each isomer as shown in Figure 2. [c] t, trace amount (less than 0.1 mg/g oil). [d] ND, not detected.

Table 4. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under the Conditions of 210 °C and 500 rpm

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil)								
	1	2 + 3	4	5	6	7	8	9	total
0	t[c]	ND[d]	ND	ND	ND	ND	ND	0.86	0.86
10	7.81	2.00	7.09	1.64	1.36	0.27	0.27	8.90	29.35
20	11.99	4.91	10.63	3.27	2.45	1.09	5.72	15.90	55.97
30	14.54	7.45	12.27	4.18	3.27	1.64	7.00	25.90	76.23
40	15.72	9.09	12.36	4.91	3.63	2.27	8.27	33.35	89.59
50	16.63	10.45	12.27	5.36	4.00	2.63	9.45	38.71	99.49
60	16.35	11.08	11.81	5.45	4.27	3.00	7.36	43.89	103.22
70	15.45	11.90	11.54	5.82	4.72	3.36	8.36	45.70	106.85
80	15.08	12.36	10.63	6.09	4.54	3.73	8.81	47.52	108.67
90	14.54	11.45	9.18	5.54	4.09	3.00	9.36	44.52	101.67
100	14.08	11.54	8.27	5.36	4.27	3.82	8.27	43.07	98.67
110	12.72	10.27	7.27	5.27	3.82	3.63	7.81	40.43	91.22
120	11.18	9.45	6.18	4.72	3.63	3.45	7.18	36.89	82.68
130	9.81	6.45	6.91	4.27	3.09	3.09	6.36	31.80	71.78
140	8.09	6.81	3.91	3.54	2.54	2.63	5.09	26.53	59.15
160	4.54	2.91	2.91	2.09	1.54	1.54	3.00	14.63	33.16

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time. [b] Identity of each isomer as shown in Figure 2. [c] t, trace amount (less than 0.1 mg/g oil). [d] ND, not detected.

not shown), which was very close to the previously reported value of 4.70 mg total CLA/g oil for butter (*17*).

The results in Tables 2, 3, and 4 showed that initially cis, trans or trans, cis isomers (peaks 1 and 4) were formed, but as the reaction time increased trans, trans isomers (peak 9) became the predominant CLA isomers in the hydrogenated soybean oil. With the hydrogenation at 210 °C and 500 rpm, the maximum quantities of 7t,9c/9c,11t/8t,10c isomers (peak 1), 10c,12t/9t,11c/11c,13t isomers (peak 4), and 10t,12t/9t,11t/8t,10t/7t,-9t isomers (peak 9) were obtained at 50, 40, and 80 min, respectively. The identity of the biologically active CLA isomers is not clearly known although it is generally assumed to be c-9,t-11 isomer (peak 1) for anticarcinogenic properties, and assumed to be t-10,c-12 isomer (peak 4) for fat partitioning activity. The contents of assumed active isomers (peaks 1 and 4) were also greatly affected by the hydrogenation temperature. The higher the hydrogenation temperature, the greater the content of active isomers formed (Tables 2−4). The

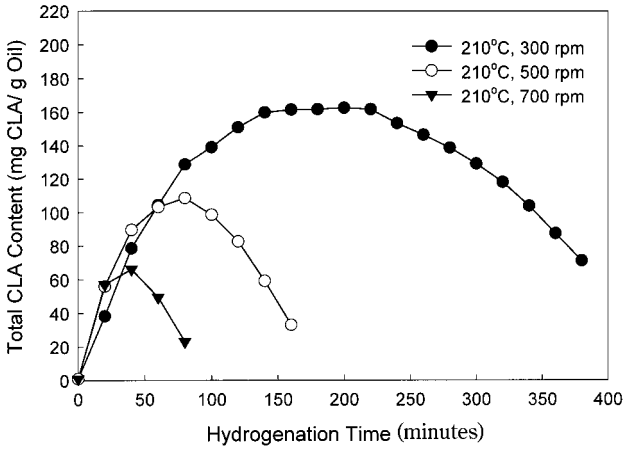

Figure 4. Effects of agitation rate on the total CLA contents in soybean oil during hydrogenation process.

Table 5. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under the Conditions of 210 °C and 700 rpm

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil)								
	1	2 + 3	4	5	6	7	8	9	total
0	t[c]	ND[d]	ND	ND	ND	ND	ND	0.86	0.86
10	6.91	2.18	7.00	2.09	1.64	1.27	0.45	10.09	31.62
20	10.72	5.45	8.90	3.45	2.54	1.27	3.18	21.53	57.06
30	10.99	6.72	8.36	3.82	2.82	1.82	4.36	27.17	66.06
40	10.36	7.00	7.36	3.91	2.73	1.91	4.72	28.17	66.15
50	8.90	6.63	5.82	3.54	2.27	1.91	4.63	26.99	60.69
60	7.09	5.45	4.63	2.63	2.00	1.64	4.00	22.08	49.52
70	5.00	4.18	2.91	2.27	1.36	0.91	3.00	16.17	35.80
80	3.18	2.18	2.18	1.45	0.91	0.82	1.82	10.54	23.08
90	1.09	0.91	0.73	0.73	0.27	0.18	0.82	5.45	10.18

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time. [b] Identity of each isomer as shown in Figure 2. [c] t, trace amount (less than 0.1 mg/g oil). [d] ND, not detected.

active isomer content in the hydrogenated soybean oil increased initially. After reaching the maximum amount, the content decreased (Tables 2−4).

Effects of Agitation Rate on the CLA Contents in Soybean Oil during Hydrogenation Process. Figure 4 shows the effects of agitation rate on the total CLA content in soybean oil during hydrogenation processes. The individual CLA isomers in soybean oil obtained during hydrogenation at 210 °C with agitation rates of 300 and 700 rpm are shown in Tables 5 and 6, respectively. As shown in Figure 4, agitation rate greatly affected the formation of CLA in soybean oil during hydrogenation. As the agitation rate decreased, the CLA formation in soybean oil increased. The maximum CLA contents in soybean oil obtained during hydrogenation at 210 °C with agitation rates of 300, 500, and 700 rpm were 162.82, 108.67, and 66.15 mg total CLA/g oil, respectively (Figure 4). That is, by decreasing the agitation rate from 700 to 300 rpm, the quantity of CLA obtained was 2.5 times higher. As the agitation rate increased, the time required to reach the maximum quantity of CLA decreased greatly. The times to reach the maximum quantity of CLA during hydrogenation under 700, 500, and 300 rpm were 40, 80, and 180 min, respectively. The formation of assumed active isomers (peaks 1 and 4) were also greatly affected by the agitation rate. The maximum contents of the isomers of peaks 1 and 4 under 700, 500, and 300 rpm were 19.62, 28.9, and 41.16 mg/ g oil, respectively (Tables

Table 6. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation under the Conditions of 210 °C and 300 rpm

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil)								
	1	2 + 3	4	5	6	7	8	9	total
0	t[c]	ND[d]	ND	ND	ND	ND	ND	0.86	0.86
10	5.72	0.91	5.82	1.18	0.73	0.27	0.36	5.09	20.08
20	9.45	1.91	9.90	2.09	1.82	0.55	1.45	11.18	38.34
40	18.08	6.91	14.72	4.54	3.27	1.45	3.63	25.99	78.59
60	20.90	10.27	16.81	5.82	4.18	2.18	5.91	38.34	104.40
80	23.53	13.72	17.63	7.00	5.27	3.18	8.18	50.34	128.84
100	23.81	15.08	16.90	7.81	1.09	3.73	8.90	61.78	139.11
120	24.71	17.26	16.26	8.63	6.09	4.54	10.72	62.88	151.10
140	24.99	18.26	15.90	9.18	6.54	5.36	12.08	67.60	159.91
160	24.90	18.44	15.26	9.18	6.63	5.82	12.54	68.78	161.55
180	24.26	18.72	14.27	9.27	6.81	6.18	12.72	70.60	162.82
200	23.90	18.90	13.36	9.36	6.91	6.54	13.90	69.87	162.73
220	23.35	19.08	12.72	9.18	7.00	6.81	13.63	70.05	161.82
240	21.72	18.17	10.99	8.63	6.81	6.81	13.63	66.69	153.46
260	20.26	17.08	10.18	8.36	6.45	6.81	13.99	63.33	146.47
280	19.17	16.26	9.09	7.09	6.36	6.81	13.63	60.42	138.83
300	18.08	14.54	8.27	7.45	6.00	6.63	12.45	55.79	129.20
320	16.08	13.36	7.36	7.00	5.63	6.27	11.54	50.88	118.12
340	13.99	11.63	6.09	6.09	5.00	5.72	10.54	44.79	103.85
360	11.99	9.72	4.91	5.18	4.27	5.00	9.00	37.25	87.32
380	9.90	8.18	3.54	4.36	3.63	4.27	7.72	29.53	71.14

[a] HT, hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time. [b] Identity of each isomer as shown in Figure 2. [c] t, trace amount (less than 0.1 mg/g oil). [d] ND, not detected.

4−6). The time to reach the maximum quantity of individual isomer also depends greatly on each isomer under the same hydrogenation condition. For example, the maximum quantity of CLA isomers of peak 1 was obtained at 140 min, but that of peak 4 was obtained at 80 min during the hydrogenation under the conditions of 210 °C and 300 rpm (Table 6).

Hydrogenation reportedly can induce the formation of various t-18:1 positional isomers, and their contents are greatly different depending on the reaction times and conditions. It has been previously reported that these trans fatty acids are associated with an increased risk of cardiovascular disease in that they behave like saturated fatty acids in humans; raising the level of low-density lipoprotein (LDL) cholesterol and decreasing the level of high-density lipoprotein (HDL) cholesterol (*30*−*33*). Thus, the overall effects of hydrogenated soybean oil on human health will depend on the hydrogenation time and conditions such as temperature and agitation rate which will determine the fatty acid composition (saturated, trans, *cis*-monunsaturated, *cis*-polyunsaturated, and conjugated linoleic acids). It is interesting to note that the partially hydrogenated soybean oil obtained after 10 min hydrogenation under 210 °C and 300 rpm of agitation rate contained considerable amounts of CLA (total 20.63 mg/g oil) with 7t,9c/9c,11t/8t,10c (5.72 mg/g oil), 10c,12t/9t,11c/11c,13t (1.00 mg/g oil), 12c,14t/10t,12c (5.82 mg), 11t,13c/9c,11c (1.36 mg/g oil), 12t,14c/10c,12c/11c,13c (1.00 mg/g oil), 12t,14t (0.27 mg/g oil), 11t,13t (0.36 mg/g oil), and 10t,12t/9t,11t/8t,10t/7t,9t isomers (5.09 mg/g oil) (Table 6). At this moment of hydrogenation, fatty acid composition of the partially hydrogenated soybean oil was not greatly different from that of the original soybean oil. Note that the iodine values of original soybean oil and soybean oils after 10 min selective hydrogenation were 132.1 and 129.3, respectively (Table 1). The fatty acid composition of original soybean oil was 10.53% palmitic acid, 4.29% stearic acid, 22.19% oleic acid, 1.33% *cis*-18:1, 0.63% unconjugated linoleic acid isomers, 52.99% linoleic acid, 0.37% arachidic acid, 1.11% linolenic acid isomers, 6.17% linolenic acids, and 0.38% behenic acid (data not shown). The fatty acid composition of hydrogenated soybean oil obtained after 10 min hydrogenation under 210 °C and 300 rpm stirring rate was 10.71% palmitic acid, 4.64% stearic acid, 1.36% t-18:1, 22.04% oleic acid, 1.32% *cis*-18:1, 4.33% unconjugated linoleic acid isomers, 46.33% linoleic acid, 0.35% arachidic acid, 1.58% linolenic acid isomers, 4.62% linolenic acids, 0.13% linolenic acid isomers, 0.37% behenic acid, and 2.21% conjugated linoleic acids (data not shown). Note that the oil hydrogenated 10 min under 210 °C and 300 rpm contained only 1.36% *trans*-18:1 fatty acid. This result showed that it can be possible to produce high-CLA-content soybean oil without major modification of fatty acid composition by a short time (10 or 20 min) selective hydrogenation under high temperature and low agitation rate conditions.

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