CLA Formation in Oils During Hydrogenation Process as Affected by Catalyst Types, Catalyst Contents, Hydrogen Pressure, and Oil Species

Mi Ok Jung[a], Jin Woo Ju[a], Dong Seong Choi[b], Suk Hoo Yoon[c], and Mun Yhung Jung[a,]*

Departments of [a]Food Science and Technology and [b]Biotechnology, Woosuk University, Jeonbuk Province 565-701, Republic of Korea, and [c]Korea Food Research Institute, Seongnam, Republic of Korea

ABSTRACT: The effects of types and amount of catalysts, hydrogen pressure, and kinds of vegetable oils on the formation of CLA isomers were studied during hydrogenation. CLA isomers were identified by using a silver ion-impregnated high-performance liquid chromatograph and 100-m cyano-capillary column gas chromatograph. A selective catalyst (SP-7) produced a considerably higher content of CLA in soybean oil than nonselective catalysts. The maximal quantity of CLA produced in soybean oil during hydrogenation increased greatly with increasing amount of catalyst. By increasing the amount of selective catalyst from 0.05 to 0.3%, the quantity of total CLA obtained was about 1.9 times higher. Changes in hydrogen pressure also greatly influenced total CLA formed. By decreasing hydrogen pressure from 0.24 to 0.024 MPa, the quantity of CLA obtained was about 1.3 times higher. With different oil species (soybean, cottonseed, and corn oils), the time to reach the maximal quantity of CLA was different under the same hydrogenation conditions. However, the maximal quantity of CLA and proportion of CLA isomers formed were almost identical, regardless of oil species tested, under the same hydrogenation conditions.

Paper no. J10035 in *JAOCS 79,* 501–510 (May 2002).

KEY WORDS: Catalysts, conjugated linoleic acids (CLA), corn oil, cottonseed oil, hydrogenation, hydrogen pressure, soybean oil.

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 (1,2), atherosclerosis (3) and non-insulin-dependent diabetes mellitus (type II diabetes) (4). CLA are reportedly also involved in the regulation of cytokine production, resulting in muscle and bone strengthening activity (5). Fat partitioning activity of CLA also has been reported, resulting in fat reduction in pigs and humans (6).

CLA are abundant in dairy products and meats from ruminant animals (7–9). Ha *et al.* (7) originally reported high levels of CLA in cheeses and milk. Chin *et al.* (8) 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).

By means of capillary GC-matrix isolation-FTIR spectroscopy, Mossoba *et al.* (10) found that *cis-trans* and *trans-cis* isomers of linoleic acid were present in hydrogenated soybean oil and margarine. Banni *et al*. (11) carried out a series of HPLC/UV/MS analyses to characterize FA 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 (mixture of hydrogenated soybean oil and palm oil) contained CLA isomers. Banni *et al.* (12) reported that a partially hydrogenated vegetable oil contained 4.24 mg total CLA/g oil. Jung and Ha (13) studied CLA formation during selective and nonselective hydrogenation processes and found that a large quantity of linoleic acids in soybean oil was formed during selective hydrogenation processes. Jung *et al.* (14) studied the effects of temperature and agitation rate on the formation CLA in soybean oil during hydrogenation and found that they greatly affected (i) the quantity of total CLA and individual isomers formed and (ii) the time to reach the maximal quantity of CLA in the partially hydrogenated soybean oil. Even though temperature and agitation rate are major variables for the hydrogenation process, other hydrogenation conditions, such as types and amounts of catalysts, hydrogen pressure and types of oils, reportedly greatly affect FA composition and physical and chemical properties of hydrogenated oils (15,16). However, the effects of types and amounts of catalyst, hydrogen pressure, and oil species on the formation of CLA in oils have never been previously reported.

Thus, the objective of this research was to study the effects of catalyst types, catalyst amount, hydrogen pressure, and oil species on the quantity of total CLA and CLA isomers in vegetable oils during hydrogenation processes.

EXPERIMENTAL PROCEDURES

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 Chemicals (Paris, KY). Sodium methoxide in

---

*To whom correspondence should be addressed at Department of Food Science and Technology, Woosuk University, Samrea-Up, Wanju-Kun, Jeonbuk Province 565-701, Republic of Korea.
E-mail: munjung@core.woosuk.ac.kr

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. Refined, bleached, and deodorized (RBD) soybean oil, cottonseed oil, and corn oil without any additives were obtained from Wellga Oil and Fat Ltd. (Seoul, Korea). The PV of these oils were less than 0.1 meq/kg oil. Selective (SP-7) and nonselective catalysts (N-222, N-325, N-545, N-655) nickel catalysts were obtained from Engelhard (Jackson, MS). Both the selective and nonselective catalysts contained reduced nickel mounted on an inert support. They were protected in fully hardened edible grade vegetable oil and were supplied in pastille form. Only SP-7 catalyst contained 1% sulfur; the others did not contain sulfur.

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 hydrogen pressure, temperature, and agitation rate controllers. The hydrogenation was carried out with a commercially available selective catalyst (SP-7; Engelhard) and nonselective catalysts (N-222, N-325, N-545, N-655; Engelhard), with different catalyst amounts (0.05, 0.1, 0.2, 0.3%), and at different hydrogen pressures (0.024, 0.049, 0.097, 0.24 MPa). The temperature and agitation rate used during the hydrogenation process were 210 ± 2°C and 500 rpm. To study the formation of CLA during hydrogenation of different oils, three different vegetable oils (corn, cottonseed, and soybean) were hydrogenated with a selective catalyst (SP-7) at 230°C, 0.024 MPa hydrogen pressure, and 300 rpm agitation rate. The oil samples (10 g each) were collected at the predetermined interval during hydrogenation.

Preparation of FAME. For extracting fats from butter and cheese, diethyl either (300 mL) was added to butter or cheese, and the mixture was stirred with a glass rod. The diethyl ether fraction was transferred to 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 undisturbed until two layers were clearly separated. The bottom water layer was discarded to remove the water-soluble components in the sample. This washing step was repeated two more times. The diethyl ether was evaporated from the collected diethyl ether layer by using a rotary vacuum evaporator at 35°C. The butter and cheese oils thus obtained 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. FAME were extracted with 2,2,4-trimethylpentane containing an internal standard (heptadecanoic acid).

Silver ion-HPLC. Silver ion-HPLC separation of CLA methyl esters was carried out using a high-performance liquid chromatograph (Shimadzu, Tokyo, Japan) equipped with a 20-μL injection loop (Waters, Milford, MA) and UV detector operated at 233 nm (17). Three ChromSpher 5 Lipids analytical silver-impregnated columns (each 4.6 mm i.d. × 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 was operated isocratically at a flow rate of 1.0 mL/min.

GC. The isolation of CLA was carried out by GC. FAME samples of 2–6 μL each were injected into a gas chromatograph equipped with an FID. The column used was a highly polar fused-silica capillary column (cyanopropyl siloxane phase, SP2380; 100 m × 0.25 mm, 0.25 μm thickness; Supelco Inc., Bellefonte, PA). A 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 the injector and detector were 230 and 250°C, respectively. The initial oven temperature of 170°C was 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 as normalized to the weight of the internal standard.

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

$$\text{CLA (mg/g)} = (A_x)(W_{\text{IS}})(\text{CF}_x)\big/(A_{\text{IS}})(W_s)(1.04) \qquad [1]$$

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), and W_s = sample weight (in g). The conversion factor 1.04 was adopted from the previous work (18) to express the results as mg FA/g fat rather than as methylesters. Since CLA isomers have identical active carbon (detector-responding carbon) numbers and numbers of unsaturations, all CLA isomers have the same theoretical detector response (18). Thus, the same correction factor for different CLA isomers was used (18). The theoretical correction factor (CF_x) was calculated by using the theoretical detector response of the internal standard (heptadecanoic acid) compared to that of CLA (14,19). The relative theoretical detector responses for the same weight of methyl esters of C17:0, C18:0, and C18:2 are 1.991:1:1.013. Thus, the calculated theoretical correction factor (CF_x) is 0.991/1.010 = 0.978.

Iodine value (IV). IV of the partially hydrogenated soybean oils were determined by AOCS official method Cd 1c-85 (20).

RESULTS AND DISCUSSION

CLA identification. Soybean oil was hydrogenated for 20 min with 0.1% of a commercially available Ni catalyst (SP-7) at 210°C and with a 500 rpm agitation rate. The resultant hydrogenated soybean oil was subjected to Ag$^+$-HPLC to isolate and identify the individual CLA isomers. Figure 1 shows the partial Ag$^+$-HPLC chromatograms of authentic CLA, cheese FAME, and hydrogenated soybean oil methylesters. The elution patterns of chromatographic peaks for the authentic CLA methylesters and cheese FAME were exactly same as those reported previously (21). The individual Ag$^+$-HPLC peak of

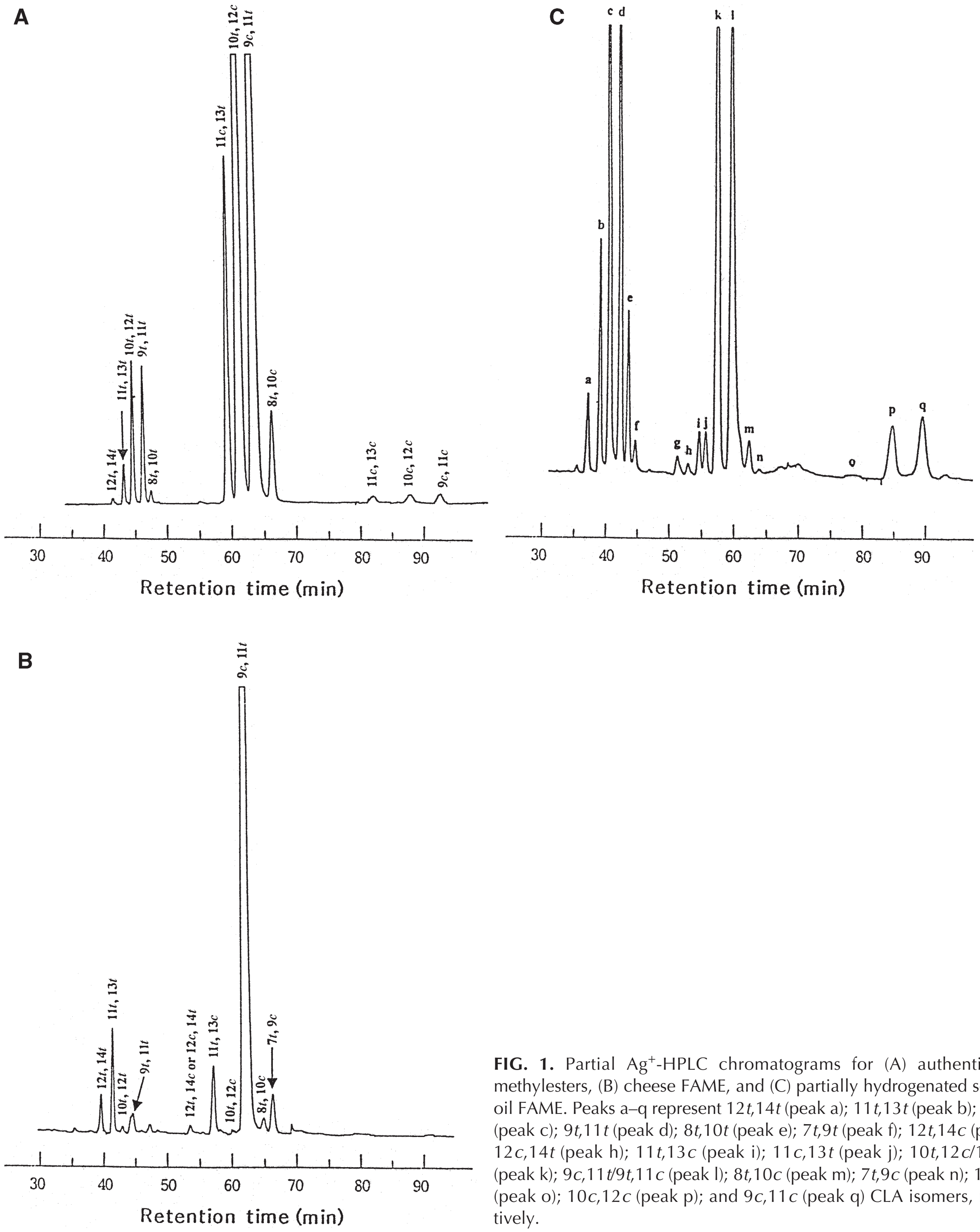

FIG. 1. Partial Ag^+-HPLC chromatograms for (A) authentic CLA methylesters, (B) cheese FAME, and (C) partially hydrogenated soybean oil FAME. Peaks a–q represent 12*t*,14*t* (peak a); 11*t*,13*t* (peak b); 10*t*,12*t* (peak c); 9*t*,11*t* (peak d); 8*t*,10*t* (peak e); 7*t*,9*t* (peak f); 12*t*,14*c*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

the hydrogenated soybean oil was identified by comparison with those of authentic CLA standards and cheese CLA that were previously reported in the literature (17,21). Two peaks (peaks **g** and **h**), which were not present in authentic CLA and cheese, were first assumed as 12*t*,14*c* and 12*c*,14*t* CLA isomers, respectively, by the expected elution order as previously reported. The identification of these isomers (peaks **g** and **h**) was reaffirmed as 12*t*,14*c* and 12*c*,14*c* isomers, respectively, by the GC analysis of the collected fractions of these Ag^+-HPLC peaks (14,17,21). The isomers of Ag^+-HPLC peaks of the hydrogenated soybean oil were identified as shown in the caption of Figure 1.

In Figure 2 are gas chromatograms of hydrogenated soybean oil and authentic CLA isomers. To identify the individual GC peaks of authentic CLA and hydrogenated soybean oil, each fraction (of the Ag^+-HPLC chromatographic peak of

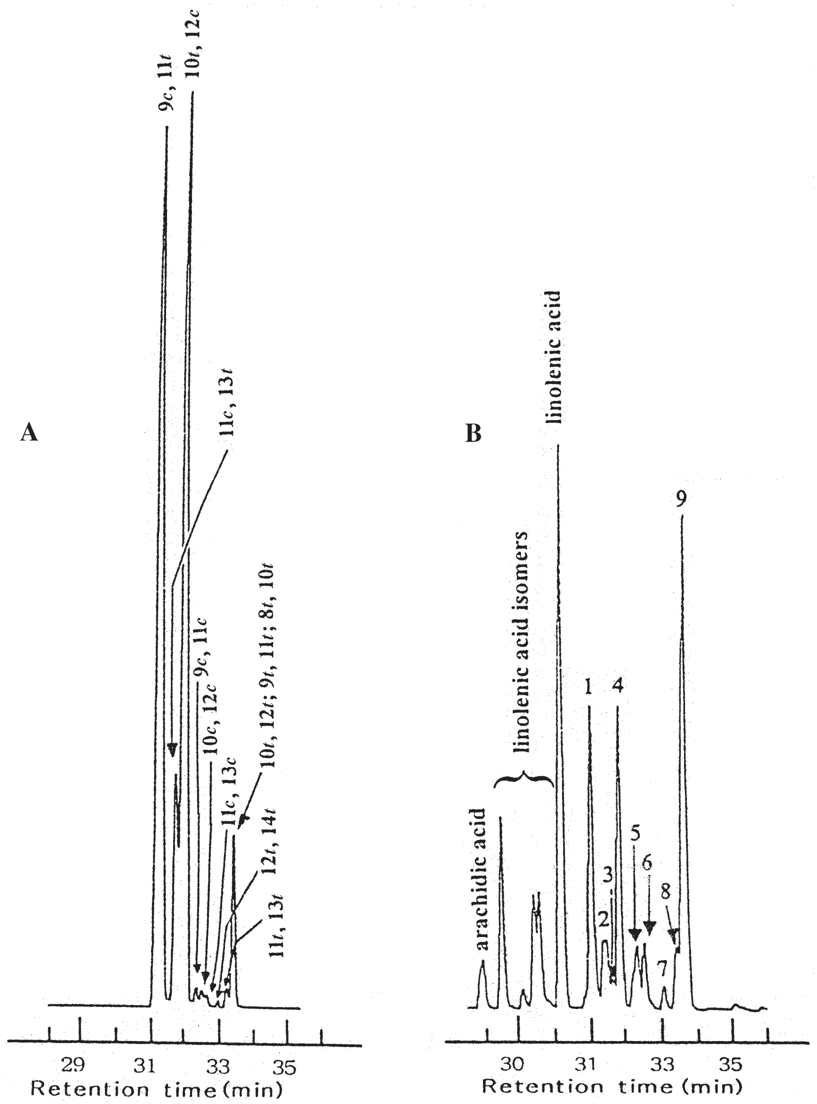

FIG. 2. Gas chromatograms of (A) authentic CLA methylesters and (B) partially hydrogenated soybean oil FAME. Peaks 1–9 represent 7*t*,9*c*/9*c*,11*t*/8*t*,10*c* (peak 1); 10*c*,12*t* (peak 2); 9*t*,11*c*/11*c*,13*t* (peak 3); 12*c*,14*t*/10*t*,12*c* (peak 4); 11*t*,13*c*/9*c*,11*c* (peak 5); 12*t*,14*c*/10*c*,12*c*/11*c*,13*c*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

authentic CLA and hydrogenated soybean oil) was collected by repeated Ag$^+$-HPLC runs, and the collected fractions were injected into the gas chromatograph. Each GC peak was identified, as shown in Figure 2. The GC elution order of the CLA isomers was exactly the same as the one expected, as suggested by Sehat *et al.* (17,21), indicating the correct identification of individual CLA isomers in hydrogenated soybean oil.

Effects of catalyst types on the CLA contents in soybean oil during the hydrogenation process. The effects of different catalysts (SP-7, N-222, N-325, N-545, and N-655) on the total CLA contents in soybean oil during hydrogenation processes under the same reaction conditions are shown in Figure 3. The hydrogenations were carried out under conditions of 0.1% catalyst, 210°C, 500 rpm agitation rate, and 0.049 MPa hydrogen pressure. The selective catalyst (SP-7) showed a much lower hydrogenation rate than nonselective-type catalysts (N-222, N-325, N-545, N-655), as was expected. IV of soybean oil hydrogenated for 60 min with SP-7, N-222, N-325, N-545, and N-655 were 112.0, 103.8, 104.6, 106.3, and 104.2, respectively, indicating much lower activity of selective catalyst SP-7 (Tables 1,2; some data not shown). Soybean oil before hydrogenation contained only 0.86 mg *trans,trans* CLA isomers (peak 9)/g oil. The CLA content in soybean oil increased initially with hydrogenation time, and after reaching a maximum, it decreased. Hydrogenation with a selective-type nickel catalyst (SP-7) produced especially high CLA contents, compared with nonselective-type nickel catalysts (N-222, N-325, N-545, N-655) (Fig. 3). The maximal CLA content obtained in soybean oil during hydrogenation with 0.1% SP-7 was 108.67 mg total CLA/g oil (Fig. 3). The nonselective catalysts (N-222, N-325, N-545,

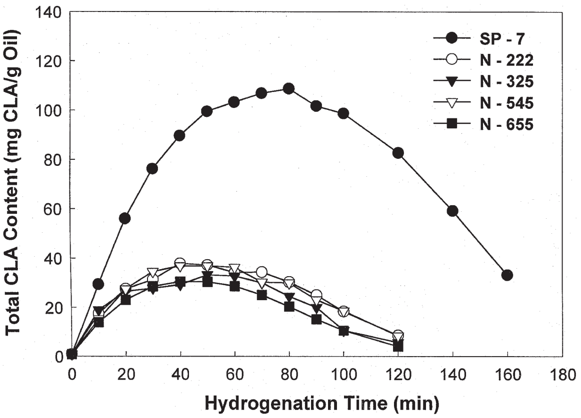

FIG. 3. Effects of different nickel catalysts on the quantity of total CLA formed in soybean oil during the hydrogenation process. The hydrogenation was performed with 0.1% catalyst under conditions of hydrogen pressure 0.049 MPa, reaction temperature 210°C, and agitation rate 500 rpm. All catalysts supplied by Engelhard (Jackson, MS); SP-7 is a selective-type catalyst; the others are nonselective.

N-655) produced much lower levels of CLA than the selective catalyst (SP-7). There were no major differences in maximal CLA contents produced with nonselective catalysts (N-222, N-325, N-545, N-655) (Fig. 3).

The selective-type Ni catalyst (SP-7) contained 1% sulfur, but the other tested nonselective Ni catalysts did not. Thus, the higher production of CLA by selective-type catalyst (SP-7) might be due to the poisoning effect of sulfur contained in the catalyst. It is generally known that sulfur-containing Ni catalysts promote the isomerization of the monoenoic FA to its *trans* $C_{18:1}$ isomers in hydrogenated vegetable oils. The present result shows that sulfur-containing Ni catalyst also promotes the isomerization of linoleic acid to its conjugated isomers.

The time to reach the maximal contents of CLA was much longer with the selective catalyst (SP-7) than with nonselective catalysts. The maximal CLA content in soybean oil was obtained after 80, 40, 50, 50, and 40 min of hydrogenation with 0.1% catalyst of SP-7, N-222, N-325, N-545, and N-655, respectively (Fig. 3). We also analyzed the CLA content of butter to check the accuracy of the determination of the CLA contents in partially hydrogenated soybean oils. Our analytical result showed that butter contained 4.58 mg total CLA/g oil (data not shown), which was very close to the previously reported value of 4.70 mg total CLA/g oil for butter (8).

The profiles of CLA isomers produced during hydrogenation differed with type of catalyst (selective- and nonselective-type). The identity of the biologically active CLA isomers is not definitively known although it is generally assumed to be *c*9,*t*11 isomer (peak 1) for anticarcinogenic properties and *t*10,*c*12 isomer (peak 4) for fat-partitioning activity. The selective-type catalyst (SP-7) initially produced *cis,trans* or *trans,cis* isomers (peaks 1,4), but as the reaction time increased *trans,trans* isomers (peaks 8,9) became the predominant CLA isomers in the hydrogenated soybean oil. The percentage of *trans,trans* isomers (peaks 8,9) reached 52.99% at 90 min hydrogenation with selective catalyst (Table 1). However, it is interesting to note that nonselective-type catalysts (N-222, N-325, N-545, N-655) produced considerably lower levels of *trans,trans* isomers (peak 9) (Table 2; some data not shown). For example, the percentage of the *trans,trans* isomers reached only 35.61% of total CLA isomers in soybean oil hydrogenated with nonselective cata-

TABLE 1
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.1% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.049 MPa, 210°C, and 500 rpm

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)										t-$C_{18:1}$[d]
(min)	1	2 + 3	4	5	6	7	8	9	Total	IV[c]	(%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	7.81	2.00	7.09	1.64	1.36	0.27	0.27	8.90	29.35	127.4	2.47
20	11.99	4.91	10.63	3.27	2.45	1.09	5.72	15.90	55.97	124.3	5.21
30	14.54	7.45	12.27	4.18	3.27	1.64	7.00	25.90	76.23	121.0	7.98
40	15.72	9.09	12.36	4.91	3.63	2.27	8.27	33.35	89.59	117.9	10.72
50	16.63	10.45	12.27	5.36	4.00	2.63	9.45	38.71	99.49	115.4	13.33
60	16.35	11.08	11.81	5.45	4.27	3.00	7.36	43.89	103.22	112.0	16.08
70	15.45	11.90	11.54	5.82	4.72	3.36	8.36	45.70	106.85	108.4	19.01
80	15.08	12.36	10.63	6.09	4.45	3.73	8.81	47.52	108.67	107.5	21.94
90	14.54	11.45	9.18	5.54	4.09	3.00	9.36	44.52	101.67	104.5	24.09
100	14.08	11.54	8.27	5.36	4.27	3.82	8.27	43.07	98.67	102.8	26.25
110	12.72	10.27	7.27	5.27	3.72	3.63	7.81	40.43	91.22	99.6	29.16
120	11.18	9.45	6.18	4.72	3.63	3.45	7.18	36.89	82.67	96.1	33.37
130	9.81	6.45	6.91	4.27	3.09	3.09	6.36	31.80	71.78	93.8	34.51
140	8.09	6.81	3.91	3.54	2.54	2.63	5.09	26.53	59.15	92.8	37.06
160	4.54	2.91	2.91	2.09	1.54	1.54	3.00	14.63	33.16	87.3	44.05

[a] HT, hydrogenation time (i.e., referring to the hydrogenated soybean oil obtained after the specified hydrogenation time). SP-7, selective catalyst (Engelhard, Jackson, MS).
[b] Identity of each isomer is shown in Figure 2.
[c] IV, iodine value.
[d] t-$C_{18:1}$, *trans*-$C_{18:1}$ FA content (%).
[e] Trace, <0.1 mg/g oil; ND, not detected.

TABLE 2
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.1% N-325 Catalyst Under Conditions of Hydrogen Pressure 0.049 MPa, 210°C, and 500 rpm[a]

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)										t-$C_{18:1}$[d]
(min)	1	2 + 3	4	5	6	7	8	9	Total	IV[c]	(%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	6.18	1.27	5.18	1.27	0.73	0.00	0.82	3.45	18.90	125.4	2.54
20	7.00	1.45	7.09	1.18	1.09	0.64	1.36	6.72	26.53	120.0	4.81
30	7.36	1.82	7.54	0.00	0.00	0.00	2.00	9.09	27.80	115.9	8.25
40	7.00	1.91	7.09	0.09	0.00	0.00	2.36	10.81	29.26	111.8	10.91
50	6.63	1.91	6.54	1.45	1.45	1.09	2.27	11.81	33.16	108.3	12.49
60	6.81	1.73	6.27	1.54	1.18	1.09	2.18	11.81	32.62	104.6	12.92
70	6.27	1.54	5.45	1.36	2.18	0.09	2.09	10.99	29.98	101.5	15.23
80	4.91	1.27	4.27	0.91	1.36	0.73	1.64	9.45	24.53	97.6	18.00
90	3.36	1.09	2.82	0.82	1.18	0.00	1.27	7.54	18.08	93.1	20.73
100	1.82	0.55	1.64	0.00	0.00	0.00	0.914	4.63	9.54	88.9	24.21

[a–e]For footnotes see Table 1. N-325, nonselective catalyst (Engelhard, Jackson, MS).

TABLE 3
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.05% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.049 MPa, 210°C, and 500 rpm[a]

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)										t-$C_{18:1}$[d]
(min)	1	2 + 3	4	5	6	7	8	9	Total	IV[c]	(%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
20	8.45	2.73	8.45	2.27	1.91	0.73	1.82	13.54	39.89	126.1	4.29
40	13.08	6.63	10.63	4.18	3.00	1.64	4.18	25.71	69.05	121.3	8.81
60	13.81	8.72	10.36	4.72	3.45	2.27	5.45	33.16	81.96	115.6	13.33
80	12.72	9.18	9.54	5.00	3.54	2.63	6.00	36.25	84.86	111.8	17.45
100	11.72	9.18	8.27	4.91	3.36	2.82	6.18	36.34	82.77	107.7	21.69
120	11.36	8.72	7.00	4.54	3.27	2.73	6.36	33.89	77.87	103.3	25.80
140	9.72	7.63	5.82	4.00	2.82	2.54	5.82	29.98	68.33	99.5	29.87
160	7.81	6.72	4.00	3.27	2.36	2.18	3.54	25.90	55.79	96.3	35.27

[a–e]For footnotes see Table 1.

lyst N-325 as shown in Table 2. At this moment of hydrogenation, the percentage of assumed active isomers (peaks 1 and 4) was 38.90%.

Effects of catalyst amount on the CLA contents in soybean oil during hydrogenation process. The effects of catalyst amount on the total CLA contents in soybean oil during hydrogenation processes are shown in Figure 4. Tables 3–5 show the contents of individual CLA isomers in soybean oil during hydrogenation with 0.05, 0.2, and 0.3% selective catalyst SP-7, respectively; data for 0.1% SP-7 are shown in Table 1. The hydrogenation was carried out under conditions of 210°C, 500 rpm agitation rate, and hydrogen pressure 0.049 MPa. As the catalyst amount increased, the hydrogenation rate increased. The drops in IV per minute with 0.05, 0.1, 0.2, and 0.3% SP-7 catalyst during the first 60 min of hydrogenation were 0.28, 0.34, 0.35 and 0.43, respectively (Tables 1,3–5). A sixfold higher catalyst load (from 0.05 to 0.3%) increased the hydrogenation rate by only 1.5 times. The result indicated that the hydrogenations were performed under conditions of gas–liquid mass transfer limitation.

The amount of catalyst during hydrogenation greatly affected the quantities produced of total CLA and CLA isomers as well as the time to reach the maximal quantity of CLA in the partially hydrogenated soybean oil. The maximal CLA levels obtained by hydrogenation of soybean oil with 0.05, 0.1, 0.2, and 0.3% catalyst were 84.86, 108.67, 137.74, and 159.28 mg CLA/g oil, respectively (Fig. 4). That is, by increasing the catalyst from 0.05 to 0.3%, the quantity of CLA obtained was 1.9 times higher. The higher catalyst content seemed to induce

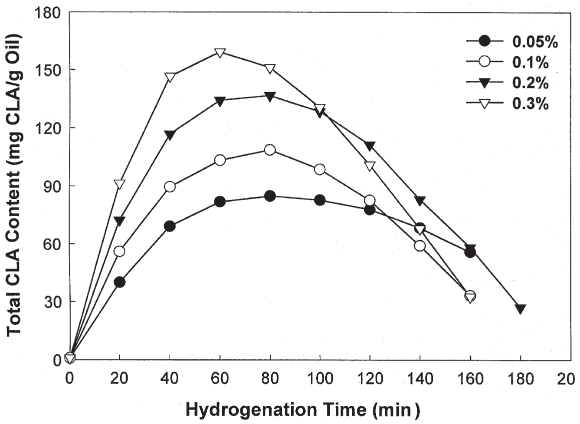

FIG. 4. Effects of catalyst amount (0.05, 0.1, 0.2, and 0.3%) on the quantity of total CLA formed in soybean oil during the hydrogenation process. The hydrogenation was performed with SP-7 catalyst under conditions of hydrogen pressure 0.049 MPa, reaction temperature 210°C, and agitation rate 500 rpm. For catalyst information see Figure 3.

TABLE 4
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.2% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.049 MPa, 210°C, and 500 rpm[a]

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)										t-$C_{18:1}$[d]
(min)	1	2 + 3	4	5	6	7	8	9	Total	IV[c]	(%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	8.81	2.27	8.81	2.45	1.73	0.55	1.45	11.07	37.16	127.8	2.63
20	14.81	6.36	13.08	4.27	3.09	1.45	3.63	25.53	72.23	124.5	5.36
30	18.35	9.54	14.90	5.72	3.91	2.27	6.27	37.71	98.67	120.4	8.58
40	19.26	11.99	15.08	6.63	4.82	3.18	8.27	47.52	116.76	116.9	11.53
50	20.72	13.63	14.63	7.27	5.27	3.82	9.09	54.15	128.57	113.9	14.27
60	19.53	14.81	13.99	7.72	5.45	4.36	10.54	57.70	134.11	111.1	17.10
70	20.53	15.36	12.99	7.81	5.72	4.91	11.18	59.24	137.74	108.8	19.72
80	18.54	15.54	11.99	7.90	5.72	5.27	11.81	59.88	136.65	104.7	22.68
90	19.17	15.36	10.81	7.63	5.72	5.45	12.18	57.79	134.11	103.5	25.76
100	17.99	14.72	9.72	7.36	5.63	5.54	11.27	56.24	128.48	100.7	28.70
110	16.90	13.90	8.54	7.00	5.36	5.54	11.08	52.88	121.21	98.0	31.53
120	15.36	12.81	7.45	6.45	4.91	5.27	10.45	48.61	111.30	95.6	34.20
130	13.54	11.36	6.54	5.91	4.63	5.09	9.63	42.80	99.49	92.9	37.03
140	7.45	7.54	5.72	5.45	4.27	5.00	8.90	38.80	83.14	90.3	40.01
160	7.09	4.82	3.54	3.82	3.09	3.54	6.45	25.90	58.24	85.9	36.78
180	1.36	2.91	1.45	1.45	0.00	1.82	3.63	14.45	27.08	82.0	50.85

[a–e]For footnotes see Table 1.

a lower hydrogen concentration near the catalyst surface and thus promote the isomerization of linoleic acid to its conjugated isomers. It has been shown that an increase in catalyst amount induces a decrease in hydrogen concentration near the catalyst surface (15,16). It has also been reported that a lower hydrogen concentration near the catalyst surface induces the formation of *trans* fat (15). The contents of isomers (peaks 1 and 4) assumed to be active were also greatly increased with the increased catalyst amount (Tables 3–5).

As the catalyst amount increased, the time to reach the maximal CLA content decreased (Fig. 4). Maximal CLA content in soybean oil was obtained after 80, 80, 70, and 60 min during hydrogenation with catalyst amounts of 0.05, 0.1, 0.2, and 0.3%, respectively. Even though the total CLA content increased with increasing amounts of catalysts, the compositions of CLA isomers in soybean oil hydrogenated with different amounts was almost identical (Tables 3–5).

Effects of hydrogen pressure on the CLA contents in soybean oil during the hydrogenation process. The effects of hydrogen pressure on the total CLA content in soybean oil during hydrogenation processes are shown in Figure 5. The individual CLA isomer contents and IV in soybean oil obtained during hydrogenation under hydrogen pressures of 0.024, 0.097 and 0.24 MPa are shown in Tables 6–8, respectively. As the hydrogen pressure increased from 0.024 to 0.24 MPa, the rate of hydrogenation increased. The IV drops per minute dur-

TABLE 5
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.3% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.049 MPa, 210°C, and 500 rpm[a]

HT[a]	CLA isomer[b] content in soybean oil (mg/g oil)										t-$C_{18:1}$[d]
(min)	1	2 + 3	4	5	6	7	8	9	Total	IV[c]	(%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	11.08	3.18	11.18	2.63	1.18	0.91	2.18	15.81	48.16	125.6	3.5
20	15.90	7.36	15.54	4.82	3.82	1.91	5.45	36.62	91.41	120.6	7.0
30	21.35	12.81	17.26	7.27	5.00	3.27	8.18	51.15	126.30	117.3	10.3
40	23.71	15.45	16.99	8.36	5.91	4.27	10.90	60.97	146.56	114.2	13.5
50	22.35	17.54	15.54	8.90	6.36	5.36	12.08	67.15	155.28	110.6	16.9
60	22.08	17.90	14.54	9.18	6.72	6.00	13.72	69.14	159.28	106.5	20.9
70	22.35	17.72	12.81	9.00	6.54	6.27	13.63	67.60	155.92	104.7	23.1
80	20.99	17.26	11.18	8.54	6.63	6.63	14.45	65.51	151.19	100.9	27.4
90	19.53	15.99	10.18	8.18	6.36	6.63	13.54	60.97	141.38	98.8	28.8
100	17.99	14.81	8.63	7.54	6.09	6.54	12.72	56.24	130.57	96.5	33.2
110	16.17	13.17	7.36	6.81	5.63	6.27	12.18	49.70	117.30	93.7	34.8
120	14.08	11.81	5.36	6.00	5.00	5.72	11.18	41.80	100.95	91.7	38.0
130	11.81	9.54	4.91	5.18	4.45	5.18	9.36	36.07	86.50	88.2	42.7
140	9.36	7.63	3.45	4.00	3.63	4.27	7.72	27.80	67.87	85.8	42.9
150	7.09	3.73	4.36	3.09	2.91	3.36	5.54	21.35	51.43	83.1	48.4

[a–e]For footnotes see Table 1.

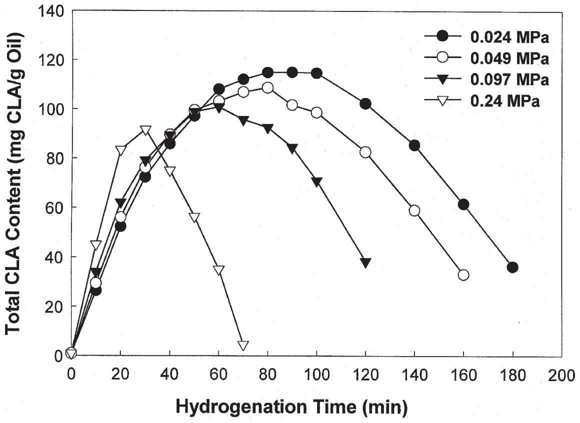

FIG. 5. Effects of hydrogenation pressure (0.024, 0.049, 0.097, and 0.24 MPa) on the quantity of total CLA formed in soybean oil during the hydrogenation process. The hydrogenation was performed with 0.1% SP-7 catalyst under conditions of reaction temperature 210°C and agitation rate 500 rpm. For catalyst information see Figure 3.

ing the first 60 min of hydrogenation at hydrogen pressures of 0.024, 0.049, 0.097, and 0.24 MPa were 0.27, 0.34, 0.36, and 0.74, respectively. Especially in the region of hydrogen pressure from 0.097 to 0.24 MPa, an increase in hydrogen pressure promoted the hydrogenation rate greatly. An increase in hydrogen pressure of 2.5 times (from 0.097 to 0.24 MPa) induced a hydrogenation rate that was 2.1 times higher. This suggested that hydrogen pressure below 0.097 MPa produced gas–liquid mass transfer limitation in the system.

Hydrogen pressure also affected the formation of CLA in soybean oil during hydrogenation (Fig. 5). As hydrogen pressure decreased, CLA formation in soybean oil increased. The maximal CLA contents in soybean oil obtained during hydrogenation at 0.024, 0.049, 0.097, and 0.24 MPa hydrogen pressure were 114.94, 108.67, 100.85, and 91.68 mg total CLA/g oil, respectively (Fig. 5). That is, by decreasing hydrogen pressure from 0.24 to 0.024 MPa, the quantity of CLA obtained was 1.3 times higher. Gas–liquid mass transfer limitation in the system apparently favors the production of CLA during hydrogenation. It is known that lower hydrogen pressure causes a decrease in dissolved hydrogen concentration in the slurry, inducing a condition of gas–liquid mass transfer limitation in the system (15,16). It has also been reported that the lower hydrogenation favored the formation of *trans* FA during hydrogenation (15). Our result also showed that lower hydrogen pressure induced a greater amount of CLA.

As hydrogen pressure increased, the time to reach to maximal quantity of CLA decreased. To reach the maximal quantity of CLA during hydrogenation under 0.024, 0.049, 0.097, and 0.24 MPa required 90, 80, 60, and 30 min, respectively. The formation of the isomers assumed to be active (peaks 1 and 4) was affected by hydrogen pressure. The maximal contents of the isomers of peaks 1 and 4 under 0.024, 0.49, 0.097 and 0.24 MPa were 24.35, 21.99, 20.98, and 19.36 mg/g oil, respectively (Tables 1, 6–8). The time to reach the maximal quantity of an individual isomer was unique to each isomer under the same hydrogenation condition. For example, the maximal quantity of CLA isomers of peak 1 was obtained at 80 min, but that of peak 4 was obtained at 90 min during the hydrogenation under the condition of 0.024 MPa hydrogen pressure (Table 6).

Effects of different vegetable oils on CLA formation during the hydrogenation process. The production of total CLA content from different vegetable oils during hydrogenation processes are shown in Figure 6. The IV of corn, cottonseed, and soybean oils were 126.7, 119.0, and 132.1, respectively (data not shown). The hydrogenation rate of soybean oil was the highest, followed by cottonseed oil and corn oil in decreasing order. The CLA maxima formed during hydrogena-

TABLE 6
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.1% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.024 MPa, 210°C, and 500 rpm[a]

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil)									IV[c]	t-$C_{18:1}$[d] (%)
	1	2 + 3	4	5	6	7	8	9	Total		
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	6.63	1.54	6.91	1.64	1.36	0.00	1.00	7.36	26.44	128.5	2.06
20	11.81	4.36	10.36	3.27	2.36	0.91	2.45	16.81	52.34	125.8	4.40
30	14.27	6.27	12.54	4.27	3.09	1.54	3.91	26.08	72.42	123.7	6.36
40	14.99	7.81	12.81	4.54	3.45	2.00	5.27	34.89	85.77	120.9	8.93
50	15.45	9.18	12.72	5.91	3.63	2.63	6.81	40.89	97.22	119.3	10.54
60	17.17	11.72	12.99	6.36	4.45	3.09	7.72	44.52	108.03	115.8	13.27
70	17.81	11.99	12.63	6.45	4.54	3.36	8.09	47.16	112.03	114.0	14.12
80	17.81	12.54	11.90	6.54	4.72	3.73	8.63	48.97	114.85	112.3	17.43
90	17.26	12.90	10.99	6.63	4.72	3.91	8.72	49.79	114.94	109.0	19.69
100	16.90	12.99	10.36	6.63	4.72	4.18	9.63	49.25	114.67	107.0	22.65
110	15.63	12.27	9.36	6.36	4.54	4.18	9.00	48.16	109.49	104.6	25.19
120	14.54	11.54	8.36	5.91	4.36	4.09	9.18	44.43	102.40	101.3	27.73
130	13.17	10.63	7.27	5.36	4.00	3.91	8.54	40.80	93.68	98.9	29.93
140	11.90	9.72	6.27	5.09	3.63	3.73	7.72	37.43	85.50	96.7	32.53
160	8.45	7.27	3.91	3.63	2.73	2.82	5.00	27.89	61.69	91.9	38.26
180	4.91	3.36	3.09	2.27	1.64	1.73	2.09	17.26	36.34	87.7	42.76

[a–e]For abbreviations see Table 1.

TABLE 7
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.1% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.097 MPa, 210°C, and 500 rpm[a]

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil) 1	2 + 3	4	5	6	7	8	9	Total	IV[c]	$t\text{-}C_{18:1}$[d] (%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	7.81	2.18	7.90	2.27	1.73	0.55	1.36	10.27	34.07	127.4	3.08
20	12.54	5.45	11.18	3.82	2.73	1.27	3.18	21.99	62.15	124.3	6.05
30	13.63	7.36	11.99	4.63	3.36	1.82	4.91	31.44	79.14	120.1	9.45
40	13.99	8.81	11.81	5.09	3.63	2.36	6.09	37.62	89.41	116.7	12.43
50	15.26	10.54	11.72	5.72	4.00	2.91	7.18	41.52	98.86	113.9	15.40
60	14.72	10.99	10.81	5.82	4.27	3.27	7.90	43.07	100.85	110.8	18.38
70	11.90	9.90	9.54	5.54	3.91	3.27	7.90	43.79	95.77	106.1	22.66
80	12.45	10.54	8.36	5.45	3.82	3.45	7.45	41.07	92.59	104.7	24.68
90	11.99	9.99	6.72	4.82	3.54	3.27	6.36	37.71	84.41	101.3	25.45
100	10.09	8.18	5.36	4.09	3.09	2.82	5.91	31.53	71.05	97.6	32.58
110	8.00	6.54	4.18	3.45	2.45	2.36	5.18	24.99	57.15	93.1	35.69
120	5.27	4.54	2.54	2.36	1.64	1.64	3.63	16.81	38.43	90.6	36.25
130	2.82	2.27	1.45	1.36	0.91	0.82	2.27	8.54	20.44	86.8	43.43

[a–e]For footnotes see Table 1.

TABLE 8
Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained During Hydrogenation with 0.1% SP-7 Catalyst Under Conditions of Hydrogen Pressure 0.24 MPa, 210°C, and 500 rpm[a]

HT[a] (min)	CLA isomer[b] content in soybean oil (mg/g oil) 1	2 + 3	4	5	6	7	8	9	Total	IV[c]	$t\text{-}C_{18:1}$[d] (%)
0	Trace[e]	ND[e]	ND	ND	ND	ND	ND	0.86	0.86	132.1	0
10	9.63	2.91	9.63	0.73	0.73	0.36	3.09	18.08	45.16	123.7	6.16
20	14.54	7.81	11.45	4.54	3.27	2.27	5.72	33.80	83.41	115.5	13.31
30	14.27	9.45	9.99	5.09	3.54	2.73	7.09	39.52	91.68	108.6	20.01
40	8.36	6.81	6.81	4.27	3.09	2.82	6.63	36.25	75.05	97.2	28.12
50	4.18	4.36	5.45	3.45	2.45	2.36	5.82	28.35	56.42	93.0	36.07
60	2.36	2.73	3.18	2.18	1.54	1.64	3.54	17.99	35.16	87.6	43.33

[a–e]For footnotes see Table 1.

tion of different vegetable oils was almost the same regardless of the type of oil. The CLA maxima in corn, cottonseed, and soybean oils obtained during hydrogenation at 230°C with an agitation rate of 300 rpm and 0.5% selective catalyst (SP-7) were 232.33, 237.24, and 233.33 mg total CLA/g oil, respectively (Fig. 6). The CLA isomer compositions of the different vegetable oils were also very similar (data not shown). The maximal contents of the isomers assumed to be active (peaks 1 and 4) in soybean, corn, and cottonseed oils were 50.16, 49.88, and 51.7 mg/g oil, respectively (data not shown). However, the times required to reach maximal contents of CLA in the oils differed with the vegetable oil (Fig. 6), i.e., for corn, cottonseed, and soybean oils the times were 140, 100, and 80 min, respectively.

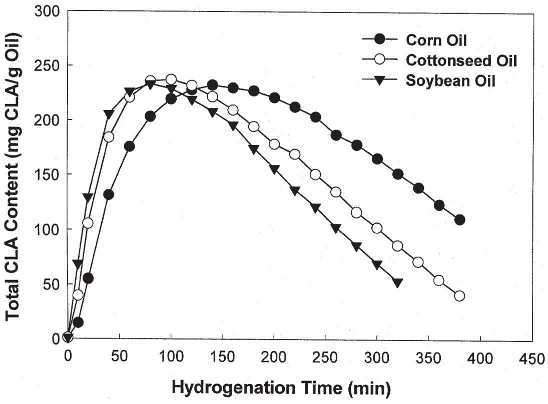

FIG. 6. Effects of different oils (corn, cottonseed, and soybean) on the quantity of total CLA formed iduring the hydrogenation process. The hydrogenation was performed with 0.5% SP-7 catalyst under conditions of reaction temperature 230°C, 0.024 MPa, and agitation rate 300 rpm. For catalyst information see Figure 3.

Hydrogenation reportedly can induce the formation of various *t*-18:1 positional isomers, and their contents depend on the reaction times and conditions (16). These *trans* FA are associated with an increased risk of cardiovascular disease in that they act like saturated FA in humans, raising the level of LDL cholesterol and decreasing the level of HDL cholesterol (22,23). Thus, the overall effects of hydrogenated soybean oil on human health depend on the hydrogenation time and conditions such as temperature and agitation rate, which determine the FA composition (saturated, *trans, cis*-monunsaturated, *cis*-polyunsaturated, and CLA). As shown in Table 5, hydrogenated soybean oil obtained after 10 min of hydrogenation with the selective-type catalyst (SP-7) under conditions of lower hydrogen pressure (0.5 kg/cm^2) and high catalyst content (0.3%) contained a high amount of CLA (48.16 mg CLA/g oil), which was about eight times greater than levels in major dietary sources for CLA such as dairy products and ruminant animal meats (8,9). After 10 min of hydrogenation the *trans*-$C_{18:1}$ FA content of this oil was only 3.5% (Table 5). This result suggested that healthful vegetable oils containing high CLA but low *trans* FA can be produced by controlling the hydrogenation times and conditions. The present result showed, for the first time, qualitative and quantitative information on CLA formation in vegetable oils during hydrogenation as affected by catalyst types and amount, hydrogen pressure, and types of oil.

ACKNOWLEDGMENT

We express our gratitude to the ARPC of the Korean Ministry of Agriculture and Forestry for their financial support.

REFERENCES

1. Ha, Y.L., N.K. Grimm, and M.W. Pariza, Anticarcinogens from Fried Ground Beef: Heat-Altered Derivatives of Linoleic Acid, *Carcinogenesis 8*:1881–1887 (1987).
2. Liew, C., H.A.J. Schut, S.F. Chin, M.W. Pariza, and R.H. Dashwood, Protection of Conjugated Linoleic Acids Against 2-Amino-3-methylimidazo[4,5-f]quinoline-induced Colon Carcinogenesis in F344 Rat; A Study of Inhibitory Mechanisms, *Ibid. 16*:3037–3043 (1995).
3. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994).
4. Houseknecht, K.L., J.P. Vanden Heuvel, S.Y. Moya-Camarena, C.P. Portocarrero, L.W. Peck, K.P. Nickel, and M.A. Belury, Dietary Conjugated Linoleic Acid Normalizes Impaired Glucose Tolerance in the Zucker Diabetic Fatty fa/fa Rat, *Biochem. Biophys. Res. Commun. 244*:678–682 (1998).
5. Cook, M.E., C.C. Miller, Y. Park, and M.W. Pariza, Immune Modulation by Altered Nutrient Metabolism: Nutritional Control of Immune-Induced Growth Depression, *Poult. Sci. 72*: 1301–1305 (1993).
6. Park, Y., J.M. Storkson, K.J. Albright, W. Liu, and M.W. Pariza, Evidence That the *trans*-10,*cis*-12 Isomer of Conjugated Linoleic Acid Induces Body Composition Changes in Mice, *Lipids 34*:235–241 (1999).
7. Ha, Y.L., N.K. Grimm, and M.W. Pariza, Newly Recognized Anticarcinogenic Fatty Acids: Identification and Quantification in Natural and Processed Cheeses, *J. Agric. Food Chem. 37*: 75–81 (1989).
8. Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha, and M.W. Pariza, Dietary Sources of Conjugated Dienoic Isomers of Linoleic Acid, a Newly Recognized Class of Anticarcinogens, *J. Food Comp. Anal. 5*:185–197 (1992).
9. Shantha, N.C., E.A. Decker, and Z. Ustunol, Conjugated Linoleic Acid Concentration in Processed Cheese, *J. Am. Oil Chem. Soc. 69*:425–428 (1992).
10. Mossoba, M.M., R.E. McDonald, D.J. Armstrong, and S.W. Page, Identification of Minor C_{18} Triene and Conjugated Diene Isomers in Hydrogenated Soybean Oil and Margarine by GC–MI–FT–IR Spectroscopy, *J. Chromtogr. Sci. 29*:324–330 (1991).
11. Banni, S., B.W. Day, R.W. Evans, F.P. Corongiu, and B. Lombardi, Liquid Chromatographic–Mass Spectrometric Analysis of Conjugated Diene Fatty Acids in a Partially Hydrogenated Fat, *J. Am. Oil Chem. Soc. 71*:1321–1325 (1994).
12. Banni, S., B.W. Day, R.W. Evans, F.P. Corongiu, and B. Lombardi, Detection of Conjugated Diene Isomers of Linoleic Acid in Liver Lipids of Rats Fed a Choline-Devoid Diet Indicates That the Diet Does Not Cause Lipoperoxidation, *J. Nutr. Biochem. 6*:281–289 (1995).
13. Jung, M.Y., and Y.L. Ha, Conjugated Linoleic Acid Isomers in Partially Hydrogenated Soybean Oil Obtained During Nonselective and Selective Hydrogenation Processes, *J. Agric. Food Chem. 47*:704–708 (1999).
14. Jung, M.O., S.H. Yoon, and M.Y. Jung, Effects of Temperature and Agitation Rate on the Formation of Conjugated Linoleic Acids in Soybean Oil During Hydrogenation Process, *Ibid. 49*: 3010–3016 (2001).
15. Coenen, J.W.E., Hydrogenation of Edible Oils, *J. Am. Oil Chem. Soc. 53*:382–389 (1976).
16. Veldsink, J.W., M.J. Bouma, N. Schoon, and A.A.C.M. Beenackers, Heterogeneous Hydrogenation of Vegetable Oils: A Literature Review, *Catal. Rev. Sci.—Eng. 39*:253–318 (1997).
17. Sehat, N., R. Rickert, M.M. Mossoba, J.K.G. Kramer, M.P. Yurawecz, J.A.G. Roach, R.O. Adlof, K.M. Morehouse, J. Fritsche, K.D. Eulitz, H. Steinhart, and Y. Ku, Improved Separation of Conjugated Fatty Acid Methyl Esters by Silver Ion–High Performance Liquid Chromatography, *Lipids 34*:407–413 (1999).
18. Shantha, N.C., E.A. Decker, and B. Henning, Comparision of Methylation Methods for the Quantification of Conjugated Linoleic Acid Isomer, *J. AOAC Int. 76*:644–649 (1993).
19. Ackman, R.G., and J.C. Sipos, Application of Specific Response Factors in the Gas Chromatographic Analysis of Methyl Esters of Fatty Acids with Flame Ionization Detectors, *J. Am. Oil Chem. Soc. 41*:377–378 (1964).
20. American Oil Chemists' Society, *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1990.
21. Sehat, N., J.K.G. Kramer, M.M. Mossoba, M.P. Yurawecz, J.A.G. Roach, K. Eulitz, K.M. Morehouse, and Y. Ku, Identification of Conjugated Linoleic Acid Isomers in Cheese by Gas Chromatography, Silver Ion High Performance Liquid Chromatography and Mass Spectral Reconstructed Ion Profiles. Comparison of Chromatographic Elution Sequences, *Lipids 33*:963–971 (1998).
22. Judd, J.T., B.A. Clevidence, R.A. Muesing, J. Wettes, M.E. Sunkin, and J.J. Podczasy, Dietary *trans* Fatty Acids: Effects of Plasma Lipids and Lipoproteins of Healthy Men and Women, *Am. J. Clin. Nutr. 59*:861–868 (1994).
23. Aro, A., M. Jauhiainen, R. Partanen, I. Salminen, and M. Mutanen, Stearic Acid, *trans* Fatty Acids, and Dietary Fat: Effects on Serum and Lipoprotein Lipids, Apolipoproteins, Lipoprotein (a), and Lipid Transfer Proteins in Healthy Subjects, *Am. J. Clin. Nutr. 65*:1419–1426 (1997).

[Received July 16, 2001; accepted February 15, 2002]