Conjugated Linoleic Acid Isomers in Partially Hydrogenated Soybean Oil Obtained during Nonselective and Selective Hydrogenation Processes

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Partially hydrogenated soybean oil samples were collected during selective and nonselective hydrogenation processes. The formation of conjugated linoleic acids (CLAs) during hydrogenation was greatly dependent on the types and duration of hydrogenation processes. During hydrogenation processes, CLA contents increased initially. After reaching maximum CLA content, the content decreased during hydrogenation. Selective hydrogenation was much more favorable for the formation of conjugated linoleic acids. With nonselective hydrogenation process, the total CLA content was a maximum (9.06 mg total CLA/g oil) at 35 min. However, with the selective hydrogenation process, the total CLA content was a maximum (98.27 mg total CLA/g oil) at 210 min. The CLA contents in some of the tested selectively hydrogenated soybean oils were among the highest ever reported in foods.

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

INTRODUCTION

Conjugated linoleic acids (CLAs) are a group of naturally occurring isomers of linoleic acid containing a conjugated double bond system. CLAs have been recognized for their ability to prevent or cure cancer (Ha et al., 1987, 1990; Pariza and Ha, 1990; Ip et al., 1991, 1996; Liew et al., 1995; Durgam and Fernandes, 1997), atherosclerosis (Lee et al., 1994; Dolye, 1998), and NIDDM (type II diabetes) (Houseknecht et al., 1998). It also has been reported that CLAs are involved in the regulation of cytokines production, resulting in muscle and bone strengthening activity (Cook et al., 1993; Chin et al., 1994; Doyle, 1998). The fat partitioning activity of CLAs also has been reported, resulting in fat reduction in pigs and human (Dugan et al., 1998; Doyle, 1998).

CLAs are abundant in dairy products and meats from ruminant animals (Ha et al., 1989; Aneja and Murthi, 1991; Chin et al., 1992; Shantha et al., 1992, 1995; Shantha and Decker, 1993). Ha et al. (1989) originally reported high levels of CLA in cheeses and milk. Chin et al. (1992) reported that dairy products (milk, butter, cheese, and yogurt) and meats from ruminant animals contained considerable amounts of CLA (ca. 3–8 mg of total CLA/(g of fat)). The authors also reported that vegetable oils contained very low levels of CLA, ranging from 0.1 mg of CLA/(g of oil) (coconut oil) to 0.7 mg of CLA/(g of oil) (safflower oil).

Mossoba et al. (1991) 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. (1994) 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 (mixture of hydrogenated soybean oil and palm oil) contained conjugated linoleic acid isomers. Banni et al. (1995) reported that a partially hydrogenated vegetable oil contained 4.24 mg of total CLA/(g of oil). The hydrogenation of vegetable oil is a complex process. The hydrogenation is performed under various conditions to produce the hydrogenated oil with different physical and chemical properties.

The major hydrogenation conditions are divided into two classifications, which are nonselective and selective hydrogenation processes. The nonselective and selective hydrogenation processes are performed in the edible oils and fats industry with commercially available nonselective and selective catalysts, respectively. In the selective hydrogenation, the conversion of diene to a monoene fatty acid occurs more selectively compared to the conversion of monene to a saturated fatty acid. It has been well-known that the fatty acid composition of oil is greatly dependent on the duration and types of hydrogenation processes. However, these previous reports did not specify the types and duration of the hydrogenation process for the tested partially hydrogenated vegetable oil. Moreover, the authors did not determine the contents of the individual CLA isomers in the partially hydrogenated vegetable oil. Even though there is accumulated evidence that a partially hydrogenated vegetable oil contained CLA, the qualitative and quantitative information on the individual CLA formed in partially hydrogenated vegetable oil during different hydrogenation processes (selective and non-

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selective) for different hydrogenation times have never been previously reported.

The objective of this research is to determine the contents of individual CLA isomers in the partially hydrogenated soybean oils obtained from two different typical hydrogenation processes (selective and nonselective hydrogenations) for different hydrogenation times.

MATERIALS AND METHODS

Materials. Authentic CLA methyl esters were purchased from Sigma Chemical Co. (St. Louis, MO). Stearic acid, oleic acid, linoleic acid, arachidic acid, and heptadecanoic acid were purchased from Sigma. HPLC grade 2,2,4-trimethylpentane was purchased from Mallinckrodt Specialty Chem. Co. (Paris, KY). Sodium methoxide in methanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Butter was purchased from a local grocery.

Hydrogenation Conditions for the Preparation of Partially Hydrogenated Soybean Oils. The nonselective and selective hydrogenations were carried out with commercially available nonselective and selective catalysts, respectively. The hydrogenation conditions (catalyst types and amount, hydrogen pressure, reaction temperature) were followed by the catalyst manufacturer's recommendations. The nonselective hydrogenation was performed with 0.17% of a commerically available nonselective Ni catalyst (Pricat 9910, Unichema) at 215 ± 5 °C and 2.5 kg/cm² hydrogen pressure. The oil samples were collected after 7, 14, 21, 28, 35, 42, and 49 min of nonselective hydrogenation. The selective hydrogenation was performed with 0.44% of selective Ni catalyst (SP 7, Engelhard) at 215 ±5 °C and 0.5 kg/cm² hydrogen pressure. The oil samples were collected after 30, 60, 120, 150, 180, 220, 250, 280, 310, and 390 min of selective hydrogenation.

Preparation of Fatty Acid Methyl Esters. For CLA analysis of butter, butter (10 g) was heated to the liquid state, and diethyl either (300 mL) was added to dissolve the butter oil. And then butter oil in diethyl ether 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 oil was used for the methyl esterification. Soybean oil samples were methyl esterified without any pretreatment. Methyl esterification of the partially hydrogenated soybean oils and butter oil was carried out with sodium methoxide (0.25N) at 70 °C for 20 min. The fatty acids of methyl ester (FAME) were extracted with 2,2,4-trimethylpentane containing an internal standard (heptadecanoic acid).

Gas Chrogmatography. The isolation of conjugated linoleic acids was carried out by gas chromatography developed by Ha et al. (1989). FAME samples of 2−6 μL each were injected into a gas chromatograph equipped with a flame ionization detector. The column used was a Suplecowax 10 fused silica capillary column (60 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 125 kPa. The temperatures of the injector, oven, and detector were 250, 220, and 250 °C, respectively. 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 milligrams per gram of oil by using the following formula:

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

Table 1. Iodine Values of the Partially Hydrogenated Soybean Oils Obtained after Nonselective and Selective Hydrogenation Processes

nonselective HT[a] (min)	IV[b]	selective HT[c] (min)	IV[b]
0	125.4	0	125.4
7	123.9	30	124.8
14	119.9	60	124.1
21	111.6	90	120.5
28	104.2	120	116.1
35	95.2	150	111.9
42	87.1	180	106.9
49	79.4	210	100.2
56	75.1	240	95.0
		300	85.5
		360	81.3

[a] Nonselective HT: nonselective hydrogenation time (min). [b] IV: iodine value. [c] Selective HT: selective hydrogenation time (min).

where A_x = peak area of CLA, A_{IS} = peak area of internal standard, CF_x = theoretical correction factor for CLA calculated on the basis of an internal standard, W_{IS} = weight of internal standard added to the sample (mg), and W_s = sample weight (g). The conversion factor 1.04 was adopted from the previous work (Shantha et al., 1993) to express the results as milligrams of fatty acid per gram of fat rather than as methyl esters. Since the different conjugated linoleic acid isomers have the same theoretical detector response, the same correction factor for different CLA isomers was used (Shantha et al., 1993). The theoretical correction factor (CF_x) was calculated by using the theoretical detector response of the internal standard (hexadecanoic acid) compared to that of conjugated linoleic acids (Ackman and Sipos, 1964). The relative theoretical detector response 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.

Gas Chromatography−Mass Spectrometry. A DS 6200 gas chromatography (Dongman, Seoul, Korea) coupled to a mass spectrometer (JMS-SX102A, JEOL) was used to determine the molecular weights of the fatty acids eluted in the gas chromatograph. Mass spectra were obtained by electron ionization at 70 eV. A column used was a Suplecowax 10 fused silica capillary column (60 m × 0.25 mm, 0.25 μm thickness). Helium was used as a carrier gas. The analytical conditions for this gas chromatography−mass spectrometry were identical to those used for the gas chromatography.

Iodine Value. Iodine values of the partially hydrogenated soybean oils were determined by AOCS official method Cd 1c-85 (AOCS, 1990).

Conjugated Dienoic Fatty Acid Content by UV Spectrometry. Conjugated dienoic fatty acids contents in the samples were measured by AOCS official method Ti 1a-64 (AOCS, 1990).

RESULTS AND DISCUSSION

CLA Identification. Soybean oil was hydrogenated by nonselective and selective hydrogenation processes. The changes in iodine values of the soybean oil during nonselective and selective hydrogenation processes are shown in Table 1. Figure 1 shows the gas chromatograms of the partially hydrogenated soybean oils obtained after a 28 min nonselective hydrogenation process (A) and after a 150 min selective hydrogenation process (B). The gas chromatography−mass spectrometry showed that peaks 1−7 have the same molecular weight of 294, indicating that all these peaks are linoleic acid isomers. The GC-MS analysis also showed that three peaks eluted just before peak 1 were found to be linolenic acid isomers. The CLA peaks were identified by both comparing the retention times of authentic CLA isomers and comparing their equivalent chain length
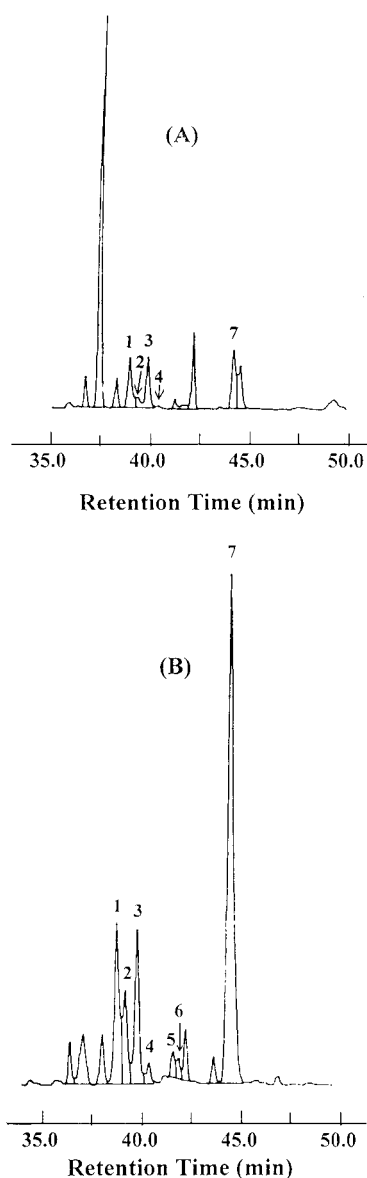
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Figure 1. Gas chromatograms of CLA methyl esters in nonselectively hydrogenated soybean oil for 28 min (A) and in selectively hydrogenated for 150 min (B). Peaks 1−7 represent c-9,t-11/c-8,t-10; c-10,t-12/t-9,c-11; t-10,c-12; c-11,t-13 or t-11,c-13; c-9,c-11/c-8,c-10; c-10,c-12/c-11,c-13; and t-9,t-11/t-10,t-12/t-8,t-10 CLA isomers, respectively.

(ECL) values of the samples and of the reference literature (Ha et al., 1989). The authentic standard CLA methyl esters (Sigma) contained 43.93% c-9,t-11/c-8,t-10 isomers, 48.77% t-10,c-12 isomer, 0.54% c-9,c-11/c-8,c-10 isomers, 0.36% c-10,c-12/c-11,c-13 isomers, and 6.40% t-9,t-11/t-10,t-12/t-8,t-10 isomers. Table 2 shows the ECL (equivalent chain length) of CLA isomers (methyl esters) on a Supelcowax 10 fused silica capillary column (60 m × 0.25 mm, 0.25 μm film thickness). ECL values of CLA methyl ester isomers were determined by plotting carbon numbers vs retention times on semilogarithmic paper as described by Miwa et al. (1960) and Ha et al. (1989). The gas chromatographic pattern for peaks 1−7 in the partially hydrogenated soybean oils was exactly the same as that for CLA identified in the cheese sample in the previous research paper (Ha et al., 1989). The calculated ECL values in the present research were comparable to the previously reported ones (Ha et al., 1989). Co-injection with the

Table 2. Equivalent Chain Length (ECL) of CLA Isomers (Methyl Esters) on a Supelcowax 10 Fused Silica Capillary Column (60 m × 0.25 mm, 0.25 μm Film Thickness)

peak no.	CLA isomer[a]	present study	Ha et al.'s Study[b]
1	c-9,t-11/c-8,t-10	19.51	19.49
2	c-10,t-12/t-9,c-11	19.56	19.53
3	t-10,c-12	19.63	19.62
4	c-11,t-13ort-11,c-13	19.71	19.67
5	c-9,c-11/c-8,c-10	19.84	19.80
6	c-10,c-12/c-11,c-13	19.88	18.82
7	t-9,t-11/t-10,t-12/t-8,t-10	20.16	20.01

[a] Peak identification was based on the previous reported works of Ha et al. (1989) and Lavillonniere et al. (1998). [b] Ha et al. (1989).

Table 3. CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during a Nonselective Hydrogenation Process

HT[a] (min)	CLA isomers[b] in partially hydrogenated soybean oil (mg/(g of oil))							
	1	2	3	4	5	6	7	total
0	t[c]	ND[d]	ND	ND	ND	ND	0.90	0.90
7	0.47	ND	0.49	ND	ND	ND	0.89	1.85
14	0.90	t	0.91	t	ND	ND	0.89	2.70
21	1.76	t	1.76	t	t	t	2.09	5.60
28	2.21	t	2.14	t	t	t	2.82	7.17
35	2.44	t	2.62	t	t	t	4.00	9.06
42	1.32	t	1.35	t	t	t	3.36	6.03
49	t	t	t	t	t	t	1.45	1.45

[a] HT: hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [b] Identity of each isomer was shown in Table 2. [c] t: trace amount (less than 0.3 mg/(g of oil)). [d] ND: not detected.

FAME of soybean oil samples and the purchased authentic CLA isomers revealed that the five peaks for c-9,t-11/c-8,t-10; t-10,c-12; c-9,c-11/c-8,c-10; c-10,c-12/c-11,c-13; and t-9,t-11/t-10,t-12/t-8,t-10 isomers from the authentic CLA were coeluted at the same retention times with peaks 1, 3, and 5−7 from the soybean oil samples, respectively. Lavillonniere et al. (1998) identified new iosmers (8,11- and 11,13-octadecadienoic acid) with all possible cis and trans configurations that coeluted with previously identified isomers by Ha et al. (1989).

By using the combined results from GC-MS, ECL, retention time, and comparison with previously reported works (Ha et al., 1989; Lavillonniere et al., 1998), it was concluded that peaks 1−7 were c-9,t-11/c-8,t-10 isomers, c-10,t-12/t-9,c-11 isomers, t-10,c-12 isomer, c-11,t-13 isomer, c-9,c-11/c-8,c-10 isomers, c-10,c-12/c-11,c-13 isomers, and t-9,t-11/t-10,t-12/t-8,t-10 isomers of CLA, respectively. It is expected that the better separation of CLA isomers in the hydrogenated soybean oil would be achieved by using a long polar capillary GC column (cyanopropyl siloxane phase column, 100 m).

CLA Contents in Partially Hydrogenated Soybean Oil Obtained during the Nonselective Hydrogenation Process. Table 3 showed the contents of CLA isomers in partially hydrogenated soybean oil obtained during nonselective hydrogenation. Soybean oil before hydrogenation contained only 0.90 mg of t-9,t-11/t-10,t-12/t-8,t-10 (peak 7) CLA isomers/(g of oil). The reaction time during the nonselective hydrogenation greatly affected the contents of the individual CLA isomers in the partially hydrogenated soybean oil. The CLA content increased initially with hydrogenation time, and after reaching a maximum, the content decreased. The total CLA content was a maximum with

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Table 4. CLA Isomers in Partially Hydrogenated Soybean Oil Obtained after Selective Hydrogenation for Different Time

HT[a] (min)	CLA isomers[b] in partially hydrogenated soybean oil (mg/(g of oil))							
	1	2	3	4	5	6	7	total
0	t[c]	ND[d]	ND	ND	ND	ND	0.90	0.90
30	4.18	t	4.84	t	0.93	0.63	5.22	15.90
60	7.52	1.87	6.71	t	1.3	0.87	12.14	30.39
90	9.07	3.01	8.19	t	1.58	1.05	21.74	44.65
120	12.65	5.85	11.44	1.29	2.00	1.41	37.38	72.00
150	13.57	7.35	12.05	1.74	2.28	1.62	46.92	85.52
180	13.49	8.05	11.36	1.98	2.53	1.63	50.97	90.00
210	13.57	9.48	10.97	2.24	2.68	1.60	57.73	98.27
240	11.09	7.61	8.37	1.95	2.19	1.36	49.28	81.85
300	3.66	2.33	2.31	0.672	t	t	11.61	20.57

[a] HT: hydrogenation time (hydrogenated soybean oil obtained after certain hydrogenation time). [b] Identity of each isomer was shown in Table 2. [c] t: trace amount (less than 0.3 mg/(g of oil)). [d] ND: not detected.

partially hydrogenated soybean oil obtained after a 35 min nonselective hydrogenation, reaching 9.06 mg of total CLA/(g of oil). The CLA in the partially hydrogenated soybean oil obtained from nonselective hydrogenation found at a detectable level were c-9,t-11/c-8,t-10 isomers (peak 1), t-10,c-12 isomer (peak 3), and t-9,t-11/t-10,t-12/t-8,t-10 isomers (peak 7) of conjugated linoleic acid. The partially hydrogenated soybean oil obtained after a 49 min nonselective hydrogenation contained only t-9,t-11/t-10,t-12/t-8,t-10 isomers (peak 7). It is interesting to note that the ratio of peak 1 (c-9,t-11/c-8,t-10 isomers) and peak 3 (t-10,c-12 isomer) was almost 1 to 1 in the nonselectively hydrogenated soybean oil. 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 4.74 mg of total CLA with 75.9% c-9, t-11/c-8,t-10 CLA isomers (peak 1) and 24.1% t-9,t-11/t-10,t-12/t-8,t-10 isomers (peak 7) (data not shown), which was very close to the previously reported value of 4.70 mg of total CLA/(g of oil) (Chin et al., 1992). This result indicated that our analytical data were precise for the determination of contents of CLA isomers. The conjugated dienoic fatty acid contents determined by UV absorption at 233 nm also increased during hydrogenation and always showed higher values than the total CLA contents obtained by gas chromatography (data not shown). For example, the conjugated dienoic fatty acid contents by UV absorption in the nonselectively hydrogenated soybean oils for 7 and 35 min were 2.10 and 12.48 mg/(g of oil), respectively (data not shown). The UV method measures total conjugated dienoic fatty acids including CLA and conjugated linolenic acids. Thus, the UV method is not a good tool for the determination of CLA contents in samples. However, these UV−analytical data are clear evidence for the conjugation reaction that occurred during the hydrogenation process.

CLA Contents in Partially Hydrogenated Soybean Oil Obtained by Selective Hydrogenation. Table 4 shows the contents of CLA isomers in partially hydrogenated soybean oil obtained during the selective hydrogenation process. The CLA contents in the selectively hydrogenated soybean oil were also greatly dependent on the hydrogenation time. The CLA content in the soybean oil reached the maximum (98.27 mg of total CLA/(g of oil)) at 210 min of the selective hydrogenation process. After that, the CLA content decreased,

resulting in no detectable amount of CLA in the partially hydrogenated soybean obtained after a 360 min selective hydrogenation. The CLA contents in some of the tested selectively hydrogenated soybean oils were among the highest ever reported in food. Note that butter oil, which is considered as a food containing a high level of CLA, contained 4.70 mg of total CLA/(g of oil) (Chin et al., 1992). It is interesting to note that selective hydrogenation was much more favorable than nonselective hydrogenation for the formation of CLA in the soybean oil. The trans, trans isomers (peak 7) were the predominant CLA isomers in the selectively hydrogenated soybean oil with still significant amounts of trans, cis isomers. The identity of the biologically active CLA isomers is not known, although it is generally assumed to be the t-9,c-11 isomer. 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 (Mensink and Katan, 1990; Troisi et al., 1992; Judd et al., 1994; Aro et al., 1997). Thus, the overall effects of hydrogenated soybean oil on human health will depend on the types and duration of hydrogenation processes, which will determine the fatty acid composition (saturated, trans, cis monounsaturated, cis polyunsaturated, and conjugated linoleic acids). It is also interesting to note that the partially hydrogenated soybean oil obtained after a 60 min selective hydrogenation produced considerable amounts of c-9,t-11/c-8,t-10 isomers (7.52 mg/(g of oil)) and c-10,t-12/t-9,c-11 isomers (1.87 mg/(g of oil)), t-10,c-12 isomer (6.71 mg/(g of oil)), c-9,c-11/c-8,c-10 isomers (1.3 mg/(g of oil)), c-10,c-12/c-11,c-13 isomers (0.87 mg/(g of oil)), and t-9,t-11/t-10,t-12/t-8,t-10 isomers (12.14 mg/(g of oil)). At this moment of hydrogenation, the fatty acid composition of the partially hydrogenated soybean oil was almost identical to that of the original soybean oil. Note that the iodine value of the original soybean oil and soybean oils after a 60 min selective hydrogenation were125.4 and 124.1, respectively (Table 1). It can be possible to produce high CLA content soybean oil without major modification of fatty acid composition by short time (30−60 min) selective hydrogenation. This present research showed, for the first time, that CLA isomers were produced in significant amounts during hydrogenation. The CLA contents were greatly dependent on the types and duration of the hydrogenation processes. Partially hydrogenated soybean oil should be included in the list of foods known to contain large amounts of CLA. Since hydrogenated soybean oils are widely consumed in the U.S. and many other nations, we strongly believe that these data would be greatly informative to both academia and the relevant food industry.

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