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Formation of Conjugated Linoleic Acids in Soybean Oil during Hydrogenation with a Nickel Catalyst As Affected by Sulfur Addition

JIN WOO JU AND MUN YHUNG JUNG†

Department of Food Science and Technology, Woosuk University, Samrea-Up, Wanju-Kun, Jeonbuk Province 565-701, Republic of Korea

The effects of sulfur addition on the formation of conjugated linoleic acid (CLA) isomers were studied during the hydrogenation of soybean oil with a nonselective type nickel catalyst. Sulfur addition greatly promoted CLA formation in soybean oil during hydrogenation. As the amount of sulfur increased to a certain level, the maximal quantity of CLA in soybean oil during hydrogenation increased greatly. However, further increase in sulfur addition above the certain level decreased CLA formation. The optimal sulfur level for the promotion of CLA formation differed greatly with the amount of nickel used. It was of great interest to find that the optimal ratio of sulfur to nickel for the promotion of CLA formation was always 0.06:1, regardless of the nickel amount used. At the same ratio of sulfur to nickel, higher nickel content induced significantly higher production of CLA ($p < 0.05$). At the optimal sulfur to nickel ratio, an increase in the nickel amount from 0.05 to 0.15% produced ~1.5 times higher levels of CLA during hydrogenation under the tested conditions. The CLA isomer compositions were greatly affected by both sulfur addition and amounts of nickel used for treatment. This is the first report of the possibility that the total quantity of CLA and their isomer composition could be manipulated during hydrogenation by controlling the amounts of sulfur and nickel.

KEYWORDS: Conjugated linoleic acids (CLA); sulfur; soybean oil; CLA isomers; gas chromatography; hydrogenation; nickel catalyst

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*, *2*), atherosclerosis (*3*), and NIDDM (type II diabetes) (*4*). It also has been reported that CLA are involved in the regulation of cytokines 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 human (*6*, *7*).

CLA are abundant in dairy products and meats from ruminant animals (*8*−*10*). Chin et al. (*9*) reported that dairy products (milk, butter, cheese, and yogurt) and meats from ruminant animals contained large quantities of CLA (~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). Banni et al. (*11*, *12*) analyzed CLA in a partially hydrogenated oil (mixture of partially hydrogenated soybean oil and palm oil) and reported that the partially hydrogenated oil contained 4.24 mg of conjugated linoleic acid/g of oil.

Jung and Ha (*13*) originally reported that the large quantity of CLA in soybean oil was formed during selective hydrogenation processes. The formation of CLA in oils was greatly affected by the reaction conditions. Jung et al. (*14*, *15*) reported that catalyst types and amounts, temperature, hydrogen pressure, and agitation rate greatly affected the quantity of total CLA and individual isomers as well as the time to reach the maximum quantity of CLA in the partially hydrogenated soybean oil. The authors concluded that the reaction condition of the gas−liquid mass transfer limitation favored the formation of CLA and that the selective type nickel catalyst greatly induced CLA formation. The selective type nickel contained sulfur; thus, it was assumed that the poisoning effect of sulfur might be responsible for the promotion of CLA formation during hydrogenation. Thus, we believed that sulfur addition could be beneficial for the production of health beneficial oil containing a large quantity of CLA. However, the qualitative and quantitative effects of sulfur addition on the formation of CLA in vegetable oils have never been previously reported.

Thus, the objective of this research was to study the qualitative and quantitative effects of sulfur addition on the quantity of total CLA and their isomer composition in soybean oils obtained during hydrogenation with nickel catalyst.

MATERIALS AND METHODS

Materials. Authentic CLA methyl esters, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester, arachidic acid methyl ester, behenic acid methyl ester, and

† Telephone 82-63-290-1438; Fax 82-63-291-9312; E-mail munjung@core.woosuk.ac.kr.

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heptadecanoic acid methyl ester were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium methoxide in methanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). RBD soybean oil without any additive was obtained from Korea Heinz Ltd. (Seoul, Korea). The peroxide value of the soybean oil was 0.2 mequiv/kg of oil. Nonselective nickel catalyst (N-545) was obtained from Engelhard (Jackson, MS). The catalyst contained reduced nickel mounted on an inert support. The catalyst was protected in fully hardened edible grade vegetable oil and was supplied in pastille form. Sulfur (powder) was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Hydrogenation of Soybean Oil. Hydrogenation was performed with a 1 L capacity hydrogenation reactor (Next Instrument, Hwa-sung, Korea) equipped with hydrogen pressure, temperature, and agitation rate controllers. Hydrogenations of soybean oils (700 g) were carried out, in duplicate, with different amounts (0.05, 0.1, and 0.15%, w/w, as percent Ni based on oil mass) of nickel catalyst (N-545, Engelhard) and different amounts of added sulfur (0−150 ppm, w/w, based on oil mass) under the following conditions: hydrogen pressure, 0.049 MPa; temperature, 220 ± 2 °C; agitation rate, 500 rpm. Ten milliliter oil samples were withdrawn from the hydrogenation reactor at the predetermined interval during hydrogenation.

Preparation of Fatty Acid Methyl Esters. Methyl esterification of oils was carried out with sodium methoxide (0.25 N) in methanol at 70 °C for 20 min. The fatty acids of methyl esters (FAME) were extracted with 2,2,4-trimethylpentane containing internal standard (heptadecanoic acid).

Gas Chrogmatography. The isolation of CLA was carried out by gas chromatography (*14*, *15*). FAME 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, 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 was 170 °C, which was held for 1 min and then increased at 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.

The CLA contents were calculated as milligrams per gram of oil by using the formula (*15*, *16*)

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

where A_x = peak area of CLA, A_{IS} = peak area of internal standard CF_x = theoretical correction factor for CLA calculated on the basis of the 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 (*16*) to express the results as milligrams of fatty acid per gram of fat rather than as methyl esters. Because the CLA isomers have identical active carbon numbers and unsaturation, all of the CLA isomers have the same theoretical detector response (*17*). Thus, same correction factor for different CLA isomers was used (*16*). The theoretical correction factor (CF_x) was calculated by using the theoretical detector response of the internal standard (hexadecanoic acid) compared to that of CLA (*14*, *17*). 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. Iodine values of the partially hydrogenated soybean oils were determined according to AOCS official method Cd 1c-85 (*18*).

Statistical Analysis. All of the data represent the means of values obtained from duplicate hydrogenation experiments. Duncan's multiple-range test was use to determine the significance of the difference at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Gas Chromatography. Figure 1 shows the partial gas chromatogram for the methyl esters of CLA in hydrogenated soybean oil. The hydrogenated soybean oil was obtained after 50 min of hydrogenation with 0.05% nickel and 30 ppm of sulfur under the following conditions: 0.049 MPa hydrogen pressure, 220 °C reactor temperature, and 500 rpm agitation rate. Identification of individual peaks was assigned on the basis of our previously published papers (*14*, *15*). The identity of each peak of the gas chromatogram was *trans*-7,*cis*-9-/*cis*-9,*trans*-11-/*trans*-8,*cis*-10- (peak 1); *cis*-10,*trans*-12- (peak 2); *trans*-9,*cis*-11-/*cis*-11,*trans*-13- (peak 3); *cis*-12,*trans*-14-/*trans*-10,*cis*-12- (peak 4); *trans*-11,*cis*-13-/*cis*-9,*cis*-11- (peak 5); *trans*-12,*cis*-14-/*cis*-10,*cis*-12/*cis*-11,*cis*-13- (peak 6); *trans*-12,*trans*-14- (peak 7); *trans*-11,*trans*-13- (peak 8); and *trans*-10,*trans*-12-/*trans*-9,*trans*-11-/*trans*-8,*trans*-10-/*trans*-7,*trans*-9-CLA (peak 9), respectively. The identities of the CLA isomers were further confirmed by the expected gas chromatographic elution order reported by Sehat et al. (*19*).

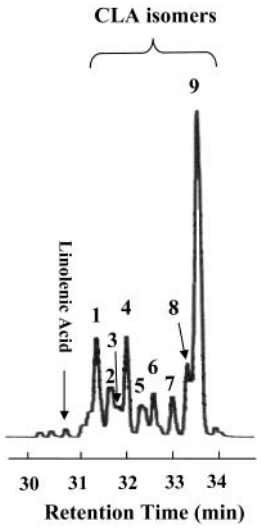

Figure 1. Gas chromatogram of partially hydrogenated soybean oil fatty acid methyl esters. The hydrogenated soybean oil was obtained after 50 min of hydrogenation with 0.05% nickel and 30 ppm of sulfur under the following conditions: hydrogen pressure, 0.049 MPa; reaction temperature, 220 °C; agitation rate, 500 rpm. Peaks: (1) *trans*-7,*cis*-9-/*cis*-9,*trans*-11-/*trans*-8,*cis*-10-CLA; (2) *cis*-10,*trans*-12-CLA; (3) *trans*-9,*cis*-11-/*cis*-11,*trans*-13-CLA; (4) *cis*-12,*trans*-14-/*trans*-10,*cis*-12-CLA; (5) *trans*-11,*cis*-13-/*cis*-9,*cis*-11-CLA; (6) *trans*-12,*cis*-14-/*cis*-10,*cis*-12/*cis*-11,*cis*-13-CLA; (7) *trans*-12,*trans*-14-CLA; (8) *trans*-11,*trans*-13-CLA; (9) *trans*-10,*trans*-12-/*trans*-9,*trans*-11-/*trans*-8,*trans*-10-/*trans*-7,*trans*-9-CLA.

Effects of Sulfur Addition on CLA Formation during Hydrogenation with 0.05% Nickel. The hydrogenation of soybean oil was carried out with 0.05% nonselective type nickel catalyst and various amounts of added sulfur under the following conditions: 0.049 MPa hydrogen pressure, 220 °C reactor temperature, and 500 rpm agitation rate (**Figure 2**). **Table 1** shows the contents of individual CLA isomers in partially hydrogenated soybean oil during hydrogenation with 30 ppm of sulfur and 0.05% nickel under the following conditions: 0.049 MPa hydrogen pressure, 220 °C reactor temperature, and 500 rpm agitation rate. Native soybean oil contained 0.8 mg of CLA/g of oil. The CLA content in soybean oil increased initially with hydrogenation time (**Figure 1**). After reaching a maximum, the CLA content decreased as hydrogenation time increased. However, the contents of *trans*-$C_{18:1}$ in soybean oil continuously increased with time during hydrogenation (**Table 1**).

Without the addition of sulfur, CLA formation was not considerable during hydrogenation with nonselective nickel catalyst (N-545). The result was consistent with a previous report (*15*). The maximal CLA content obtained in soybean oil during hydrogenation without the addition of sulfur was 40.7 mg of

Table 1. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation with 30 ppm of Sulfur and 0.05% Nickel Catalyst under the Following Conditions: Hydrogen Pressure, 0.049 MPa; Reactor Temperature, 220 °C; Agitation Rate, 500 rpm[a]

	CLA isomer content in soybean oil[b] (mg/g of oil)										
HT[c] (min)	1	2 + 3	4	5	6	7	8	9	total	IV[d]	*t*-$C_{18:1}$[e] (%)
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8 ± 0.1	132.8 ± 0.3	0.0 ± 0.0
10	11.9	0.0	13.6	0.0	1.9	0.0	1.2	10.7	39.3 ± 6.5	126.8 ± 0.1	1.9 ± 0.3
20	18.6	7.3	15.1	4.3	3.5	1.9	4.7	24.6	80.0 ± 0.3	122.5 ± 0.8	4.3 ± 1.0
30	22.1	9.0	18.2	5.7	4.8	3.1	7.1	37.1	107.1 ± 2.7	114.7 ± 1.5	8.0 ± 0.4
40	23.1	9.1	20.0	6.5	5.6	4.0	8.0	46.8	123.1 ± 1.8	113.3 ± 0.3	10.9 ± 0.6
50	23.3	10.0	19.7	7.3	6.0	4.7	10.3	51.7	133.0 ± 0.6	108.2 ± 1.6	14.5 ± 0.6
60	22.0	10.5	17.6	7.3	6.1	5.3	11.1	53.0	132.9 ± 1.9	103.4 ± 1.8	19.2 ± 1.4
70	15.4	8.7	15.0	7.0	5.8	5.6	10.7	55.1	123.3 ± 6.3	99.9 ± 3.6	23.8 ± 1.2
80	15.1	7.8	12.7	6.1	5.4	5.3	10.6	49.5	112.5 ± 5.5	95.4 ± 2.7	26.3 ± 0.4
90	12.8	7.2	10.6	5.3	5.1	4.9	9.9	44.2	100.0 ± 6.1	91.2 ± 0.63	31.1 ± 2.1
100	9.8	5.0	6.0	2.2	4.1	4.2	8.2	36.6	76.1 ± 6.6	87.0 ± 0.0	35.4 ± 1.7
110	7.6	2.9	6.0	3.4	3.4	3.5	6.6	28.0	61.4 ± 3.6	85.1 ± 0.1	38.2 ± 0.4
120	3.0	0.0	0.0	0.0	0.4	0.4	4.8	16.5	25.1 ± 5.3	80.6 ± 1.6	44.3 ± 1.7

[a] All values are means of data obtained from duplicate hydrogenation. [b] Identities of CLA isomers are shown in **Figure 1**. [c] Hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [d] Iodine value. [e] *trans*-$C_{18:1}$ fatty acid content (%).

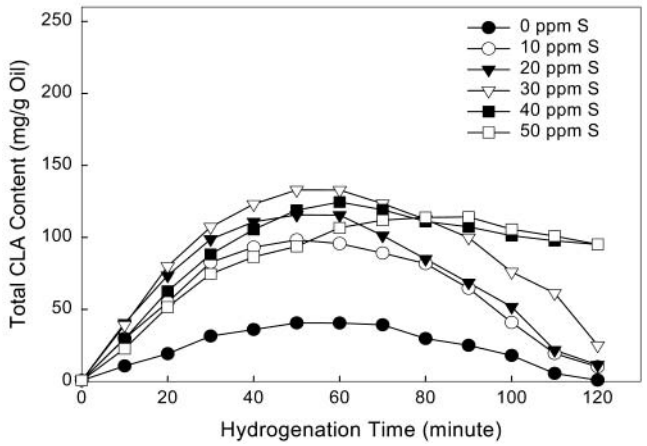

Figure 2. Effects of different levels of sulfur addition on the quantity of total CLA formed in soybean oil during the hydrogenation process. Hydrogenation was performed with 0.05% nickel catalyst under the following conditions: hydrogen pressure, 0.049 MPa; reaction temperature, 220 °C; agitation rate, 500 rpm. The iodine value drops of the 0, 10, 20, 30, 40, and 50 ppm sulfur treatment groups during the first 60 min of hydrogenation were 0.39, 0.45, 0.50, 0.49, 0.41, and 0.36 IV/min, respectively.

CLA/g of oil (**Figure 2**). The addition of sulfur greatly promoted the formation of CLA during hydrogenation. The higher production of CLA with sulfur addition might result from its poisoning effect. It is well-known that sulfur greatly reduces catalyst activity and induces the isomerization of monoenoic fatty acid to its *trans*-$C_{18:1}$ isomers in the hydrogenation of vegetable oils (*20*−*22*). It has been proposed that the poisoning effect of sulfur is due to its interaction with catalyst by electron donation to the unoccupied d orbitals of the catalyst metal (*23*). The present research showed that sulfur addition also promoted the isomerization of linoleic acid to its conjugated isomers. However, it is of great interest to note that sulfur addition above the 30 ppm level did not further increase CLA formation. Instead, the 40 ppm sulfur addition produced a significantly lower maximal content of CLA in soybean oil than 30 ppm sulfur addition during hydrogenation with 0.05% nickel catalyst ($p < 0.05$) (**Figure 2**). The maximal CLA content in soybean oil formed at the 50 ppm sulfur addition was also significantly lower than that formed at the 40 ppm sulfur addition during hydrogenation with 0.05% nickel catalyst ($p < 0.05$). The maximal CLA contents obtained during hydrogenation with 0, 10, 20, 30, 40, and 50 ppm sulfur additions were 40.7, 98.3, 115.7, 133.0, 124.6, and 114.2 mg of CLA/g of oil, respectively. The results suggested that there was an optimal ratio of sulfur to nickel for CLA formation in soybean oil during hydrogenation. Excessive sulfur addition seemed to induce too much interaction between the sulfur and the catalyst, resulting in lost activity as a catalyst for the conjugation reaction. The 10, 20, 30, 40, and 50 ppm sulfur additions at the 0.05% nickel catalyst concentration represent nickel to sulfur ratios of 0.02:1, 0.04:1, 0.06:1, 0.08:1 and 0.1:1, respectively. The optimal ratio of sulfur to nickel for the production of CLA in soybean oil was 0.06:1.

There was no difference in time to reach maximum CLA content in soybean oil with different amounts of sulfur treatment from 0 to 30 ppm. However, sulfur addition above the 30 ppm level delayed the time to reach maximum contents of CLA. Maximum CLA content in soybean oil hydrogenated with ≤30 ppm level of sulfur addition was obtained after 50 min of hydrogenation. However, maximum CLA contents in soybean oil hydrogenated with 40 and 50 ppm sulfur additions were obtained after 60 and 90 min of hydrogenation, respectively.

The profiles of CLA isomers in oils produced during hydrogenation differed greatly with differing hydrogenation times (**Table 1**). The identity of the biologically active CLA isomers is not clearly known, although it is generally assumed to be the *cis*-9,*trans*-11 isomer (peak 1) for anticarcinogenic properties and the *trans*-10,*cis*-12 isomer (peak 4) for fat partitioning activity (*6*, *24*, *25*). Recently, however, it has been reported that other types of CLA isomers also have a biological activity (*26*). In that study, the *cis*-9,*trans*-11-CLA isomers showed inhibition efficacy of prostaglandin H synthase activity, which is involved in tumor growth in numerous systems, followed by *trans*-10,*cis*-12, *trans*-9,*trans*-11, *cis*-9, and *cis*-11 isomers, in decreasing order (*26*). Native soybean oil contained only the *trans*,*trans* isomer as shown in **Table 1**. Initially *cis*-, *trans*-, or *trans*,*cis* isomers (peaks 1 and 4) were produced during hydrogenation as shown in **Table 1**, but as the reaction time increased, *trans*,*trans* isomers (peaks 7−9) became the predominant CLA isomers. The percentages of *trans*,*trans* isomers (peaks 7−9) of total CLA in soybean oils obtained after 10 and 50 min of hydrogenations with 30 ppm sulfur addition were 30.3 and 50.1%, respectively (**Table 1**). The CLA isomer profiles were also greatly affected by the addition of sulfur. Hydrogenation with no sulfur addition induced greatly lower *trans*,*trans* isomer proportions. The percentage of *trans*,*trans*

Table 2. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation with 60 ppm of Sulfur and 0.1% Nickel Catalyst under the Following Conditions: Hydrogen Pressure, 0.049 MPa; Reactor Temperature, 220 °C; Agitation Rate, 500 rpm[a]

	CLA isomercontent in soybean oil[b] (mg/g of oil)										
HT[c] (min)	1	2 + 3	4	5	6	7	8	9	total	IV[d]	*t*-$C_{18:1}$[e] (%)
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8 ± 0.1	132.8 ± 0.3	0.0 ± 0.0
10	17.0	2.3	17.1	0.0	5.1	0.0	3.2	16.7	61.4 ± 1.2	125.2 ± 0.2	2.1 ± 0.0
20	25.3	7.1	23.2	5.8	4.8	3.0	6.8	36.0	112.0 ± 2.3	121.2 ± 1.0	4.6 ± 0.4
30	29.1	10.5	25.7	7.8	6.6	4.7	10.1	52.8	147.3 ± 1.1	115.0 ± 1.3	8.8 ± 0.1
40	30.0	12.5	25.2	9.1	7.6	6.0	12.6	64.4	167.4 ± 1.2	110.1 ± 0.9	12.4 ± 0.0
50	28.5	15.4	20.4	9.3	8.1	7.0	16.0	67.3	172.0 ± 2.7	106.2 ± 0.7	16.6 ± 0.2
60	26.5	12.9	20.1	8.8	8.2	7.4	15.3	66.8	166.0 ± 0.2	101.0 ± 1.1	21.3 ± 1.1
70	23.4	11.9	16.8	8.1	7.5	7.4	14.0	62.6	151.7 ± 1.8	96.8 ± 0.4	25.8 ± 0.8
80	19.9	10.0	13.7	7.1	6.8	6.9	12.9	54.4	131.7 ± 2.9	92.6 ± 0.4	29.2 ± 0.8
90	15.7	7.7	10.5	5.6	5.7	5.8	10.3	43.6	104.9 ± 2.2	88.1 ± 0.1	35.6 ± 0.1
100	11.7	0.3	5.7	1.7	4.0	4.2	7.1	34.5	69.2 ± 9.5	82.9 ± 0.4	40.2 ± 1.4
110	4.9	0.0	1.6	0.0	1.0	2.6	4.6	22.4	37.0 ± 9.4	78.7 ± 1.3	44.9 ± 2.3
120	0.0	0.0	0.0	0.0	0.0	0.0	1.4	6.5	7.9 ± 4.6	75.4 ± 0.6	50.4 ± 1.5

[a] All values are means of data obtained from duplicate hydrogenation. [b] Identities of CLA isomers are shown in **Figure 1**. [c] Hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [d] Iodine value. [e] *trans*-$C_{18:1}$ fatty acid content (%).

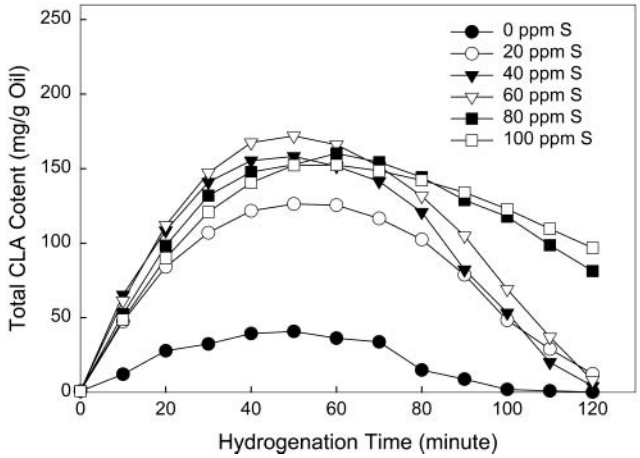

Figure 3. Effects of different levels of sulfur addition on the quantity of total CLA formed in soybean oil during the hydrogenation process. Hydrogenation was performed with 0.1% nickel catalyst under the following conditions: hydrogen pressure, 0.049 MPa; reaction temperature, 220 °C; agitation rate, 500 rpm. The iodine value drops of the 0, 20, 40, 60, 80, and 100 ppm sulfur treatment groups during the first 60 min of hydrogenation were 0.44, 0.49, 0.53, 0.53, 0.48, and 0.46 IV/min, respectively.

(peaks 7−9) of total CLA produced was only 37.3% after 50 min of hydrogenation with 0 ppm of sulfur and 0.05% nickel (data not shown).

Effects of Sulfur Addition on CLA Formation during Hydrogenation with 0.10% Nickel. The effects of sulfur addition on the total CLA contents in soybean oil during hydrogenation with 0.10% nickel under the conditions of 0.049 MPa hydrogen pressure, 220 °C reactor temperature, and 500 rpm agitation rate are shown in **Figure 3**. **Table 2** shows the contents of individual CLA isomers in partially hydrogenated soybean oil during hydrogenation with 60 ppm of sulfur and 0.1% nickel under the same conditions. During hydrogenation with 0.1% nickel, as the sulfur addition increased from 0 to 60 ppm, the formation of CLA greatly increased (**Figure 3**). However, sulfur addition above 60 ppm did not further increase the formation of CLA. Instead, addition of sulfur above the 60 ppm level decreased CLA formation during hydrogenation with 0.10% nickel catalyst. The maximum contents of CLA formed with 0, 20, 40, 60, 80, and 100 ppm sulfur additions were 40.8, 126.3, 158.3, 171.9, 160.3, and 152.5 mg of CLA/g of oil, respectively. The maximum content of CLA formed with a 60 ppm sulfur addition was significantly higher than that with others ($p < 0.05$). It is interesting to note that the promotion of CLA formation seemed to be related to the ratio of sulfur to nickel. With 0.05% Ni catalyst, 30 ppm of sulfur was optimal for the production of CLA formation. With 0.1% Ni catalyst, 60 ppm of sulfur was optimal for the production of CLA formation. The 60 ppm nickel and 0.1% nickel treatment represents a ratio of sulfur to nickel of 0.06:1.

The percentage of *tran*,*trans* CLA isomers (peaks 7−9) of the total CLA in soybean oil obtained after 50 min of hydrogenation with 60 ppm of sulfur and 0.1% nickel was 52.5% (**Table 2**). At the same ratio of sulfur to nickel, hydrogenation with 0.1% nickel induced a higher amount of *t*-$C_{18:1}$ isomer than that with 0.05% nickel (**Tables 1** and **2**).

Effects of Sulfur Addition on CLA Formation during Hydrogenation with 0.15% Nickel. To check whether the optimal sulfur to nickel ratio of 0.06 was true for the production of CLA during hydrogenation even with the higher amount of nickel treatment, hydrogenations with 0.15% Ni and various amounts of sulfur were carried out (**Figure 4**).

Without sulfur, increased nickel content from 0.05 to 0.15% did not promote CLA formation in soybean oil. At no added sulfur, the maximum CLA contents in partially hydrogenated soybean oil during hydrogenation with 0.05, 0.1, and 0.15% nickel were 40.7, 40.7, and 37.4 mg/g of oil, respectively (**Figures 1**−**3**). With 0.15% nickel catalyst, the incremental sulfur addition from 0 to 90 ppm induced higher productions of CLA. However, addition of sulfur above 90 ppm did not further increase the maximum content of CLA formed in soybean oil. The maximum CLA contents obtained during hydrogenation with 0.15% nickel by addition of 0, 30, 60, 90, 120, and 150 ppm of sulfur were 38.4, 129.4, 177.6, 196.7, 188.4, and 165.3 mg of CLA/g of oil, respectively. The result showed that 90 ppm of sulfur was an optimal amount of sulfur for the promotion of CLA formation during hydrogenation with 0.15% nickel catalyst. The sulfur to nickel ratio with 90 ppm of sulfur and 0.15% nickel is 0.06:1. This result clearly showed that the optimal ratio of sulfur to nickel for the production of CLA during hydrogenation of soybean oil was always 0.06:1, regardless of the amount of nickel tested. At the same ratio of sulfur to nickel, a higher nickel content induced a significantly higher production of CLA ($p < 0.05$). At the sulfur to nickel ratio of 0.06:1, the maximum contents of CLA formed in soybean oil during hydrogenation with 0.05, 0.1, and 0.15% nickel were 133.0, 171.9, and 196.7 mg of CLA/g of oil,

Table 3. Contents of Individual CLA Isomers in Partially Hydrogenated Soybean Oil Obtained during Hydrogenation with 90 ppm of Sulfur and 0.15% Nickel Catalyst under the Following Conditions: Hydrogen Pressure, 0.049 MPa; Reactor Temperature, 220 °C; Agitation Rate, 500 rpm[a]

	CLA isomer content in soybean oil[b] (mg/g of oil)										
HT[c] (min)	1	2 + 3	4	5	6	7	8	9	total	IV[d]	*t*-$C_{18:1}$[e] (%)
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8 ± 0.1	132.8 ± 0.3	0.0 ± 0.0
10	21.5.	6.2	18.4	3.9	3.3	0.9	4.9	11.5	70.7 ± 6.5	124.0 ± 0.1	2.6 ± 0.2
20	30.1	10.1	26.2	7.0	6.0	3.7	8.9	44.8	136.7 ± 3.6	119.5 ± 0.2	5.9 ± 0.1
30	33.4	12.8	28.4	9.3	7.7	5.7	12.5	63.4	173.3 ± 1.6	113.4 ± 0.1	8.7 ± 0.8
40	33.2	17.2	24.4	10.3	8.7	7.1	15.5	72.9	189.4 ± 3.2	108.1 ± 1.0	12.9 ± 0.1
50	31.9	15.5	24.8	10.7	9.4	8.4	16.0	80.0	196.7 ± 0.6	104.7 ± 0.4	17.2 ± 0.8
60	28.9	14.7	21.2	10.3	9.3	8.9	17.0	76.9	187.3 ± 2.9	100.7 ± 0.8	21.2 ± 0.4
70	25.0	12.6	17.8	8.9	8.9	8.7	15.9	69.0	166.8 ± 0.1	95.6 ± 0.5	25.8 ± 1.2
80	21.4	10.8	14.3	7.7	8.0	8.1	14.3	60.6	145.3 ± 4.8	91.7 ± 1.1	30.2 ± 1.3
90	13.6	6.4	14.0	5.9	6.4	6.7	11.7	45.8	110.5 ± 6.4	87.4 ± 1.1	35.9 ± 1.6
100	11.6	3.7	8.6	4.0	4.8	4.8	7.9	31.8	77.3 ± 7.5	83.6 ± 2.0	41.3 ± 2.1
110	8.3	2.3	4.0	2.3	2.9	2.9	5.6	23.5	55.7 ± 7.0	79.1 ± 1.7	44.0 ± 0.1
120	0.0	0.0	0.0	0.0	0.0	0.0	2.7	11.4	14.1 ± 1.1	74.7 ± 0.1	50.0 ± 0.6

[a] All values are means of data obtained from duplicate hydrogenation. [b] Identities of CLA isomers are shown in **Figure 1**. [c] Hydrogenation time in minutes (hydrogenated soybean oil obtained after certain hydrogenation time). [d] Iodine value. [e] *trans*-$C_{18:1}$ fatty acid content (%).

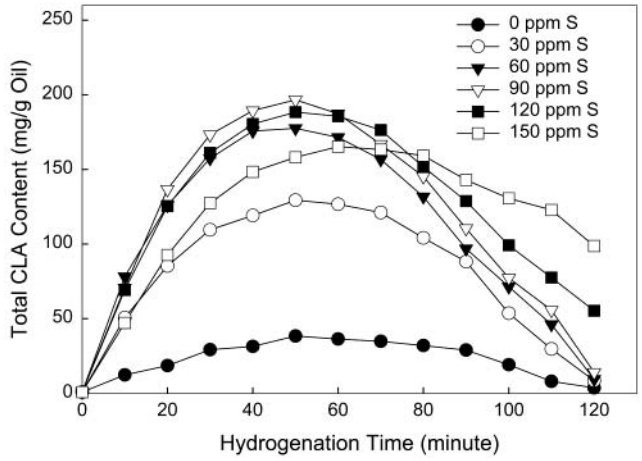

Figure 4. Effects of different levels of sulfur addition on the quantity of total CLA formed in soybean oil during the hydrogenation process. Hydrogenation was performed with 0.15% nickel catalyst under the following conditions: hydrogen pressure, 0.049 MPa; reaction temperature, 220 °C; agitation rate, 500 rpm. The iodine value drops of the 0, 30, 60, 90, 120, and 150 ppm sulfur treatment groups during the first 60 min of hydrogenation were 0.46, 0.48, 0.51, 0.54, 0.52, and 0.45 IV/min, respectively.

respectively (**Tables 1**−**3**). That is, by increasing the nickel amount from 0.05 to 0.15%, a ∼1.5 times higher level of CLA was formed during hydrogenation under the tested condition.

At the optimal ratio of sulfur to nickel (0.06:1), the higher nickel contents induced higher contents of *t*-$C_{18:1}$ and higher percentages of *trans*,*trans* isomers (peaks 7−9) in oils (**Tables 1**−**3**). The percentage of *trans*,*trans* isomers of the total CLA in soybean oil obtained after 50 min of hydrogenation with 90 ppm of sulfur and 0.15% nickel was 53.0% (**Table 3**). Note that the percentage of *trans*,*trans* isomers of total CLA in soybean oil obtained after 50 min of hydrogenation with 30 ppm of sulfur and 0.05% nickel was 50.1% (**Table 1**). The results clearly suggested that the total quantity of CLA, their isomer profiles, and *t*-$C_{18:1}$ could be manipulated by controlling the sulfur addition and nickel amount. It is also interesting to note that the partially hydrogenated soybean oil obtained after 10 min of hydrogenation with 90 ppm of sulfur and 0.15% Ni contained total CLA of 70.7 mg/g of oil, which is considered to be an exceptionally high quantity of CLA in foods. Note that the CLA content in dairy foods is ∼3−8 mg/g (*8*). At this moment of hydrogenation, the *trans*,*trans* CLA isomers (peaks 7−9) consisted of only 24.5% of total CLA, and the *t*-$C_{18:1}$ content was 2.6% (**Table 3**). The results clearly suggested the possibility of healthy beneficial soybean oil production by short-time (10 min) hydrogenation with Ni catalyst along with sulfur addition.

In summary, the present result showed, for the first time, that sulfur addition greatly promoted CLA formation in vegetable oil during hydrogenation. The optimal ratio of sulfur to nickel for the promotion of conjugated linoleic acid formation during hydrogenation was found to be 0.06:1. The optimal sulfur to nickel ratio was constant, regardless of the amount of nickel used. At the same ratio of sulfur to nickel, the higher nickel contents induced higher CLA formation in oil during hydrogenation. The CLA isomer compositions and *t*-$C_{18:1}$ were also greatly affected by the sulfur addition and hydrogenation time.

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LITERATURE CITED

(1) Ha, Y. L.; Grimm, N. K.; Pariza, M. W. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* **1987**, *8*, 1881−1887.

(2) Liew, C.; Schut, H. A. J.; Chin, S. F.; Pariza, M. W.; Dashwood, R. H. 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. *Carcinogenesis* **1995**, *16*, 3037−3043.

(3) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(4) Houseknecht, K. L.; Vanden Heuvel, J. P.; Moya-Camarena, S. Y.; Portocarrero, C. P.; Peck, L. W.; Nickel, K. P.; Belury, M. A. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker Diabetic Fatty fa/fa Rat. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 678−682.

(5) Cook, M. E.; Miller, C. C.; Park, Y.; Pariza, M. W. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult. Sci.* **1993**, *72*, 1301−1305.

(6) Park, Y.; Storkson, J. M.; Albright, K. J.; Liu, W.; Pariza, M. W. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **1999**, *34*, 235−241.

(7) Riserus, U.; Berglund, L.; Vessby, B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of metabolic syndrome: a randomized controlled trial. *Int. J. Obesity* **2001**, *25*, 1129−1135.

(8) Ha, Y. L.; Grimm, N. K.; Pariza, M. W. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J. Agric. Food Chem*. **1989**, *37*, 75−81.

(9) Chin, S. F.; Liu, W.; Storkson, J. M.; Ha, Y. L.; Pariza, M. W. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal*. **1992**, *5*, 185−197.

(10) Shantha, N. C.; Decker, E. A.; Ustunol, Z. Conjugated linoleic acid concentration in processed cheese. *J. Am. Oil Chem. Soc*. **1992**, *69*, 425−428.

(11) Banni, S.; Day, B. W.; Evans, R. W.; Corongiu, F. P.; Lombardi, B. Liquid chromatographic-mass spectrometric analysis of conjugated diene fatty acids in a partially hydrogenated fat. *J. Am. Oil Chem. Soc*. **1994**, *71*, 1321−1325.

(12) Banni, S.; Day, B. W.; Evans, R. W.; Corongiu, F. P.; Lombardi, B. 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*. **1995**, *6*, 281−289.

(13) Jung, M. Y.; Ha, Y. L. Conjugated linoleic acid isomers in partially hydrogenated soybean oil obtained during nonselective and selective hydrogenation processes. *J. Agric. Food Chem*. **1999**, *47*, 704−708.

(14) Jung, M. O.; Yoon, S. H.; Jung, M. Y. Effects of temperature and agitation rate on the formation of conjugated linoleic acids in soybean oil during hydrogenation process. *J. Agric. Food Chem*. **2001**, *49*, 3010−3016.

(15) Jung, M. O.; Ju, J. W.; Choi, D. S.; Yoon, S. H.; Jung, M. Y. CLA formation in oils during hydrogenation process as affected by catayst type, catalyst contents, hydrogenation pressure, and oil species. *J. Am. Oil Chem. Soc*. **2002**, *79*, 501−510.

(16) Shantha, N. C.; Decker, E. A.; Henning, B. Comparison of methylation methods for the quantification of conjugated linoleic acid isomer. *J. AOAC Int.* **1993**, *76*, 644−649.

(17) Ackman, R. G.; Sipos, J. C. 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.* **1964**, *41*, 377−378.

(18) AOCS. *Official Methods and Recommended of the American Oil Chemists' Society*, 4th ed.; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 1990.

(19) Sehat, N.; Kramer, J. K. G.; Mooba, M. M.; Yurawecz, M. P.; Roach, J. A. G.; Eulitz, K.; Morehouse, K. M.; Ku, Y. 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* **1998**, *33*, 963−971.

(20) Veldsink, J. W.; Bouma, M. J.; Schoon, N.; Beenackers, A. A. C. M. Heterogeneous hydrogenation of vegetable oils: A literature riview. *Catal. Rev.-Sci. Eng*. **1997**, *39*, 253−318.

(21) Drozdowski, B.; Zajac, M. Effect of concentration of some nickel catalyst poisons in oils on the course of hydrogenation. *J. Am. Oil Chem. Soc*. **1977**, *54*, 595−599.

(22) Hastert, R. C. *Hydrogenation in Bailey's Industrial Oil and Fat Products*, 5th ed.; Hui, Y. H., Ed.; Wiley: New York, 1996; Vol. 4, pp 213−300.

(23) Klimmek, H. Influence of various catalyst poisons and other impurities on fatty acid hydrogenation. *J. Am. Oil Chem. Soc*. **1984**, *61*, 200−204.

(24) Gavino, V. C.; Gavino, G.; Leblanc, M. J.; Tuchweber, B. An isomeric mixture of conjugated linoleic acids but not pure *cis*-9,*trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr*. **2000**, *130* (Suppl.), 27−29.

(25) Aldof, R. O.; Copes, L. C.; Walter, E. L. Changes in conjugated linoleic acid composition within samples obtained from a single source. *Lipids* **2001**, *36*, 315−317.

(26) Bulgarella, J. A.: Patton, D.; Bull, A. W. Modulation of prostanglandin H synthesis activity by conjugated linoleic acid (CLA) and specific CLA isomers. *Lipids* **2001**, *36*, 407−412

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