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Analysis and formation of *trans* fatty acids in hydrogenated soybean oil during heating

W.H. Liu, B. Stephen Inbaraj, B.H. Chen *

Department of Nutrition and Food Science, Fu Jen University, Taipei 242, Taiwan

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Abstract

Hydrogenated oil has been widely used for production of shortenings or margarine, however, the presence of *trans* fatty acids may be detrimental to human health. The objectives of this study were to develop an improved method for analysis of *trans* fatty acids and evaluate their formation in both unhydrogenated and hydrogenated soybean oil during heating at 160, 180 and 200 °C for varied length of time. Results showed that among the four columns tested, an Agilent HP-88 column (100 × 0.25 mm I.D., 0.2-μm film thickness) could resolve eight *trans* fatty acids and nine cis fatty acids simultaneously within 31 min with injector temperature 240 °C, detector temperature 250 °C, and column temperature 170 °C in the beginning, maintained for 24 min, increased to 220 °C at 7.5 °C/min, 230 °C at 10 °C/min, and maintained for 5 min. The contents of both *cis* and *trans* fatty acids showed a decreased trend for the increase of heating time or temperature. No *trans* fatty acid formation was observed even after extensive heating of unhydrogenated and hydrogenated soybean oil for 24 h. This phenomenon demonstrated that *trans* fatty acids can only be formed under severe conditions.

Keywords: *Trans* fatty acid; Hydrogenated soybean oil; GC–MS; Heating

1. Introduction

Fats and oils are one of the major nutrients in the diet to maintain human health by providing body energy and essential fatty acids such as linoleic acid (Frankel, 1998). Because of presence of two isolated double bonds, linoleic acid is susceptible to oxidation or degradation during heating (Chen, Tai, Chen, & Chen, 2001). In an attempt to enhance the stability of unsaturated fatty acids in edible oils, the hydrogenation process has been often employed for production of shortenings or margarine (Kris-Etherton, 1995). However, the isomerization of *cis* to *trans* fatty acids can occur during hydrogenation and result in a wide distribution of *trans* fatty acids in bakery and fried products (Aro et al., 1998; Romero, Cuesta, & Sánchez-Muniz, 2000). Although reported data on *trans* fatty acid contents in food products can be varied from one country to another, the food made with hydrogenated fats such as cookies and other bakery products, have been shown to be the main source of *trans* fatty acid in the diet (Vicario, Griguol, & Leon-Camacho, 2003).

Epidemiological studies have revealed that the intake of *trans* fatty acids in excess may raise the cholesterol level in blood (Mensink & Katan, 1990, 1993), and the concentration of low density lipoprotein in the plasma could be elevated following the consumption of hydrogenated fat containing high levels of *trans* fatty acids (Han et al., 2002). Several authors also reported that *trans* fatty acids may adversely affect the inflammatory process in atherosclerosis by increasing the peripheral blood mononuclear cell production of inflammatory cytokines (Libbey, Rid, & Maseri, 2002; Taubes, 2002). More recently, Kummerow et al. (2004) demonstrated that *trans* fats inhibit the metabolic conversion of linoleic acid to arachidonic acid and to other polyunsaturated fatty acids, a risk factor in the development of coronary heart disease.

* Corresponding author. Tel.: +886 2 29053626; fax: +886 2 29021215.
E-mail address: nutr1007@mails.fju.edu.tw (B.H. Chen).

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In view of the impact of *trans* fatty acids on human health, the quantitation of *trans* fatty acids in heated oil and food products is extremely important. The analysis of *trans* fatty acids has been previously achieved by high-performance liquid chromatography (HPLC) (Adlof, 1994; Adlof, Copes, & Emken, 1995; Christie & Breckenridge, 1989). However, the resolution of *trans* fatty acids remains poor. Juanéda (2002) used two C-18 columns to separate *trans* isomers of oleic acid in milk by HPLC, but this method fails to resolve *trans* forms of linoleic acid and linolenic acid. To remedy the problem, several authors have used gas chromatography (GC) to analyze *trans* fatty acids instead (American Oil Chemists' Society, 1990; Juanéda, 2002). Based on a GC method developed by American Oil Chemists' Society (1990), a total of 21 fatty acids, including 15 *cis* fatty acids, 1 *trans* oleic acid, 3 *trans* linoleic acids and 2 *trans* linolenic acids were separated within 37 min by using a SP-2340 column (60 m × 0.25 mm I.D., 0.2-μm film *thickness*) containing 100% polybiscyanopropyl siloxane as stationary phase. Nevertheless, the resolution is inadequate since several peaks of *trans* fatty acids are overlapped. Juanéda (2002) developed a GC method to separate 18 fatty acids in milk, including 10 *trans* fatty acids, and the major drawback is that the separation time is lengthy (50 min) and only 4 *trans* fatty acids are adequately resolved. Since many published reports still encounter difficulties in separating *cis* and *trans* fatty acids simultaneously, it is imperative to develop a precise method to determine *cis* and *trans* fatty acids in food products. Moreover, starting January 1, 2006, the US Food and Drug Administration issued a rule that the *trans* fatty acid content should be declared in the nutrition label of conventional foods and dietary supplements (Food & Drug Administration, 2003). The objectives of this study were to develop a GC method for analysis of *trans* fatty acids in unhydrogenated and hydrogenated soybean oil during heating.

2. Materials and methods

2.1. Materials

All fatty acid standards, including lauric acid methyl ester (C12:0), myristic acid methyl ester (C14:0), palmitic acid methyl ester (C16:0), stearic acid methyl ester (C18:0), arachidic acid methyl ester (C20:0), palmitoleic acid methyl ester (C16:1, Δ9 *cis*), oleic acid methyl ester (C18:1, Δ9 *cis*), linoleic acid methyl ester (C18:2, Δ9 *cis*, Δ12 *cis*), linolenic acid methyl ester (C18:3, Δ9 *cis*, Δ 12 *cis*, Δ15 *cis*), internal standard heptadecanoic acid methyl ester (C17:0), 9-*trans*-hexadecenoic acid methyl ester (C16:1, Δ9 *trans*), 6-*trans*-octadecenoic acid methyl ester (C18:1, Δ6 *trans*), 9-*trans*-octadecenoic acid methyl ester (C18:1, Δ9 *trans*), 11-*trans*-octadecenoic acid methyl ester (C18:1, Δ 11 *trans*), 9-*trans*-12-*trans*-octadecadienoic acid methyl ester (C18:2, Δ9 *trans*, Δ12 *trans*), 9-*cis*-11-*trans*-octadecadienoic acid methyl ester (C18:2, Δ9 *cis*, Δ11 *trans*) and 10-*cis*-12-*trans*-octadecadienoic acid methyl ester (C18:2, Δ 10 *cis*, Δ12 *trans*), were from Nu-Chek-Prep Inc. (Elysian, MN, USA), and 9,12,15-*trans*-octadecatrienoic methyl ester (C18:3, Δ9 *trans*, Δ12 *trans*, Δ15 *trans*) was from Sigma (St. Louis, MO, USA).

The analytical-grade solvents such as *n*-hexane, methanol and chloroform were from Merck Co. (Darmstadt, Germany). Chemicals, including potassium hydroxide, anhydrous sodium sulfate, sodium chloride and boron fluoride (in methanol) were from Riedel-de Häen Co. (Seelze, Germany). Deionized water was obtained using a Milli-Q water purification system from Millipore Co. (Bedford, MA, USA). Unhydrogenated soybean oil was from Chia-Hsin Chemical Co. (Taichung, Taiwan), while hydrogenated soybean oil was from Nan-Chiao Chemical Co. (Taoyuan, Taiwan).

2.2. Heating of oil

A 5-l unhydrogenated or hydrogenated soybean oil was poured into an oil tank separately, which was preheated to 160, 180 and 200 °C, and the heating time started to count for 4, 8, 12, 16, 20 and 24 h. The temperature-controlled oil tank (model B503) was from I-Seng Scientific Co. (Taipei, Taiwan). After the desired heating time was reached, the oil tank was cooled immediately to room temperature and a 10-ml oil sample was collected and poured into a 40-ml brown vial for storage at −20 °C. Both fresh unhydrogenated and hydrogenated soybean oil were used as control samples to compare with heated samples. Duplicate experiments were carried out for each temperature and heating time, and a total of 42 treatments were used.

2.3. Preparation of fatty acid methyl esters

A modified method based on Vicario et al. (2003) was used. A 0.5-g oil sample was mixed with 10-ml methanolic potassium hydroxide solution (0.5 N), and the mixture was saponified at 90 °C in a water bath for 10 min. After cooling to room temperature, a 8-ml BF_3-CH_3OH solution was added and the mixture was standing in a water bath at 90 °C for 5 min to promote formation of methyl ester. Again, the mixture was cooled to room temperature, then 8-ml hexane was added and the solution was heated in a water bath at 90 °C for 3 min to allow complete esterification of fatty acids. After cooling to room temperature, the saturated saline solution was added to terminate the reaction. The solution was allowed to settle until two layers were formed, and the supernatant was collected, followed by addition of 0.2-g anhydrous sodium sulfate to remove excessive moisture and evaporation of the solution to dryness. The residue was dissolved in 10-ml hexane and 1-μl was injected into GC.

2.4. GC analysis of fatty acid methyl esters

Initially, four GC capillary columns were compared with respect to the separation efficiency of standards of five saturated fatty acid methyl esters, four unsaturated fatty acid

methyl esters, eight *trans* fatty acid methyl esters and internal standard (C17:0). In addition, the various injector, column and detector temperatures, as well as flow rate and split ratio were also evaluated. The GC instrument (model 6890) equipped with flame ionization detector (FID) and mass spectrophotometer (model 5973) was from Agilent Technologies (Palo Alto, CA, USA). All the standards were dissolved in hexane to a concentration of 100 μg/ml each and stored at −20 °C until use. The characteristics of each GC column are listed below: (1) the DB-1 column (60 m × 0.32 mm I.D., 0.25-μm film thickness, coated with 100% dimethylpolysiloxane) was from J & W Scientific Co. (Folsom, CA, USA); (2) the INNOWAX column (30 m × 0.32mm I.D., 0.25-μm film thickness, coated with 100% polyethylene glycol) was from Agilent Technologies (Palo Alto, CA, USA); (3) the INNOWAX column (60 m × 0.32 mm I.D., 0.25-μm film thickness, coated with 100% polyethylene glycol); (4) the HP-88 column (100 m × 0.25 mm I.D., 0.2-μm film thickness, coated with 88% cyanopropyl-methylaryl polysiloxane) was also from Agilent Technologies.

The various *cis* and *trans* fatty acids in the oil were identified by comparing retention time and mass spectra of unknown peaks with reference standards and cochromatography with added standards. For GC–MS, the interface temperature was 270 °C with an electron multipler voltage 70 eV and ion voltage 1360 V, and detection was performed by total ion mode with a scanning range of 35–500 and rate at 2.94 scans/s. Eight concentrations of each fatty acid standard (0.5, 0.8, 1.2, 1.5, 2.0, 3.0, 4.0 and 5.0 ppm) was prepared in hexane and the detection limit was calculated based on $S/N \geqslant 3$, whereas the quantitation limit was based on $S/N \geqslant 10$. For quantitation, eight concentrations (5, 10, 20, 40, 60, 100, 150 and 250 ppm) of oleic acid methyl ester (C18:1, Δ9 *cis*) and linoleic acid methyl ester (C18:2, Δ9 *cis*, Δ12 *cis*) were prepared and mixed with internal standard (C17:0) for a final concentration of 91 ppm. Likewise, eight concentrations of the other fatty acid standards (5, 10, 20, 40, 60, 80, 100 and 150 ppm) were prepared and mixed with internal standard for a concentration of 91 ppm. Then the standard curves were obtained by plotting concentration ratio against area ratio, and the correlation coefficient (r^2) was calculated with the linear equations used for quantitation. The amount of each fatty acid in the oil was calculated based on the following formula:

$$W = \frac{\frac{A/\mathrm{RRF}}{A_\mathrm{i}} \times W_\mathrm{i}}{W_\mathrm{s}} \div \text{recovery}$$

where relative response factor $(\mathrm{RRF}) = (A/Ai) \times (W_\mathrm{i}/W)$; W is the concentration (mg/g) of each fatty acid in the oil sample; A is the peak area of each fatty acid standard; A_i is the peak area of internal standard; W_i is the concentration of internal standard; and W_s is the weight of the sample.

The recovery was accomplished by adding two concentrations (10,000 and 20,000 ppm) of 1-ml of each fatty acid standard to 0.5 g oil for extraction, with the exception of C12:0, C18:2 (Δ9 *cis*, Δ12 *trans*) and C18:2 (Δ9 *cis*, Δ 11 *trans*), because these three fatty acids were found not present in heated oil. The recovery of each fatty acid was calculated based on the ratio of the amount of each standard obtained after and before GC.

2.5. Statistical analysis

All the experiments were performed in duplicate and the data were subjected to analysis of variance using ANOVA and Duncan's multiple range test for comparison of significant difference ($P < 0.05$) using SAS (2003).

3. Results and discussion

3.1. GC analysis of cis and trans fatty acid standards

Initially, the official method published by the American Oil Chemists' Society (American Oil Chemists' Society, 1990) was adopted for separation of *cis* and *trans* fatty acid standards. However, the resolution of *trans* fatty acids remains poor, and thus a modified method was developed. Four GC capillary columns differing in length and polarity of stationary phase as described in Section 2 were evaluated, and the various GC separation conditions were also compared. After numerous studies, an Agilent HP-88 column was found to be the most appropriate for simultaneous separation of *trans* and *cis* fatty acids. For the other three columns, only 9 *cis* fatty acid standards, including C12:0, C14:0, C16:1 (Δ9*c*), C16:0, C18:2 (Δ9*c*Δ12*c*), C18:3 (Δ9*c*Δ 12*c*Δ15*c*), C18:1 (Δ9*c*), C18:0 and C20:0 were separated by using a DB-1 column. Likewise, a total of 15 fatty acids, including 6 more *trans* fatty acids were separated using an INNOWAX column (30 or 60 m). The difference is that a 60-m INNOWAX column resulted in a much longer retention time than a 30-m INNOWAX column, and several *trans* fatty acids were overlapped for both columns. The GC chromatogram of fatty acid methyl ester standards using a HP-88 column is shown in Fig. 1. A total of 18 peaks, including 5 *cis* saturated fatty acids, 4 *cis* unsaturated fatty acids, 8 *trans* fatty acids and internal standard (C17:0) were resolved within 31 min, with helium as carrier gas and flow rate at 3 ml/min, injector temperature at 240 °C and detector temperature at 250 °C. The split ratio was 10:1 and the column temperature was programmed as follows: 170 °C in the beginning, maintained for 24 min, raised to 220 °C at 7.5 °C/min, 230 °C at 10 °C/min and maintained for 5 min. With the exception of *trans* isomers of oleic acid (peaks 7, 8 and 9), all the other fatty acids were adequately resolved. We have to point out here that the partial overlap of *trans* oleic acid should not affect quantitation because, for food labeling, these three isomers can be regarded as those representing the total amount of *trans* oleic acid. Nevertheless, when compared to some other previous reports (American Oil Chemists' Society, 1990; Juanéda, 2002), this method is much better

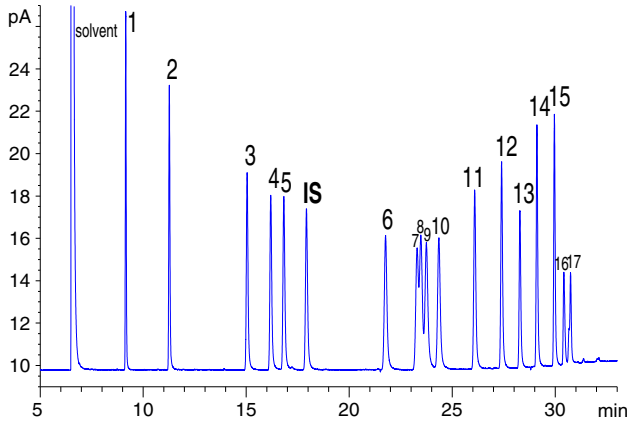

Fig. 1. GC chromatogram of FAMEs standards using a HP-88 column. Helium was used as carrier gas. The oven temperature was programmed as follows: 170 °C in the beginning, maintained for 24 min, increased to 220 °C at 7.5 °C/min, to 230 °C at 10 °C/min, maintained for 5 min. *Peaks*: (1) lauric acid methyl ester, (2) myristic acid methyl ester, (3) palmitic acid methyl ester, (4) 9-*trans*-hexadecenoic acid methyl ester, (5) palmitoleic acid methyl ester, (6) stearic acid methyl ester, (7) 6-*trans*-octadecenoic acid methyl ester, (8) 9-*trans*-octadecenoic acid methyl ester, (9) 11-*trans*-octadecenoic acid methyl ester, (10) oleic acid methyl ester, (11) 9-*trans*-12-*trans*-octadecadienoic acid methyl ester, (12) linoleic acid methyl ester, (13) 9,12,15-*trans* octadecatrienoic acid methyl ester, (14) arachidic acid methyl ester, (15) linolenic acid methyl ester, (16) 9-*cis*, 11-*trans*-octadecadienoic acid methyl ester, (17) 10-*cis*, 12-*trans*-octadecadienoic acid methyl ester. IS = internal standard.

in terms of retention time and separation number of *trans* fatty acids.

Table 1 shows the quality control data of 14 fatty acid standards. Three fatty acid standards, namely, C12:0, C18:2 (Δ9 *cis*, Δ12 *trans*) and C18:2 (Δ9 *cis*, Δ11 *trans*) were excluded because they were not detected in heated soybean oil. Both intra- and inter-day tests are routinely used for evaluation of precision of the developed analytical method, which are often carried out by comparing the concentration difference of multiple analyses within one day and between days, and the concentration difference should be as minimal as possible to attain a high reproducibility for this method (International Conference on Harmonization, 1996). The coefficient of variation (CV) (%) of the intra-day variability based on the mean concentration of five injections within one day ranged from 0.2% to 2.7% while the CV of the inter-day variability based on the mean concentration of five injections every week for a total of 5 weeks ranged from 1.0% to 3.6%. This result clearly indicated a high reproducibility was achieved by this method. Table 2 shows the detection limit (DL) and quantitation limit (QL) of 14 fatty acid standards. The DL based on $S/N \geqslant 3$ ranged from 0.8 to 1.2 ppm, whereas the QL based on $S/N \geqslant 10$ ranged from 2.6 to 3.9 ppm. These values were lower than a report by Ruiz-Jimenez, Priego-Capote, and Luque de Castro (2004), who determined the amount of *trans* fatty acids in bread and found the DL ranged from 0.98 to 3.93 ppm and the QL ranged from 3.23 to 12.98 ppm. The r^2 of all the linear regression equations of 14 standard curves were higher than 0.99. Table 3 shows

Table 1
Quality control data of 14 fatty acid methyl esters standards analyzed by GC

Fatty acid methyl ester standard	Intra-day[a] variability	Inter-day[b] variability
	CV (%)	CV (%)
Myristic acid methyl ester (C14:0)	1.7	2.4
Palmitic acid methyl ester (C16:0)	0.2	1.0
9-*trans*-Hexadecenoic acid methyl ester (C16:1,9*t*)	2.1	3.2
Palmitoleic acid methyl ester (C16:1,9*c*)	2.7	3.6
Stearic acid methyl ester (C18:0)	0.2	1.8
6-*trans*-Octadecenoic acid methyl ester (C18:1,6*t*)	0.5	2.3
9-*trans*-Octadecenoic acid methyl ester (C18:1,9*t*)	0.7	1.7
11-*trans*-Octadecenoic acid methyl ester (C18:1,11*t*)	0.8	1.8
Oleic acid methyl ester (C18:1,9*c*)	0.7	1.4
9-*trans*-12-*trans*-Octadecadienoic acid methyl ester (C18:2,9*t*12*t*)	1.6	2.0
Linoleic acid methyl ester (C18:2,9*c*12*c*)	1.5	2.2
9,12,15-*trans*-Octadecatrienoic acid methyl ester (C18:3,9*t*12*t*15*t*)	0.4	2.0
Arachidic acid methyl ester (C20:0)	0.7	2.5
Linolenic acid methyl ester (C18:3,9*c*12*c*15*c*)	0.7	1.6

[a] Mean concentration of five injections within one day.
[b] Mean concentration of five injections every week for a total of five weeks.

Table 2
Detection and quantitation limits of 14 fatty acid methyl esters standards analyzed by GC

Fatty acid methyl ester standard	DL (ppm)[a]	QL (ppm)[b]
Myristic acid methyl ester (C14:0)	0.8	2.6
Palmitic acid methyl ester (C16:0)	0.8	2.6
9-*trans*-Hexadecenoic acid methyl ester (C16:1,9*t*)	1.2	3.9
Palmitoleic acid methyl ester (C16:1,9*c*)	0.8	2.6
Stearic acid methyl ester (C18:0)	0.8	2.6
6-*trans*-Octadecenoic acid methyl ester (C18:1,6*t*)	0.8	2.6
9-*trans*-Octadecenoic acid methyl ester (C18:1,9*t*)	0.8	2.6
11-*trans*-Octadecenoic acid methyl ester (C18:1,11*t*)	0.8	2.6
Oleic acid methyl ester (C18:1,9*c*)	0.8	2.6
9-*trans*-12-*trans*-Octadecadienoic acid methyl ester (C18:2,9*t*12*t*)	0.8	2.6
Linoleic acid methyl ester (C18:2,9*c*12*c*)	1.2	3.9
9,12,15-*trans* Octadecatrienoic acid methyl ester (C18:3,9*t*12*t*15*t*)	1.2	3.9
Arachidic acid methyl ester (C20:0)	1.2	3.9
Linolenic acid methyl ester (C18:3,9*c*12*c*15*c*)	1.2	3.9

[a] DL: limit of detection based on $S/N \geqq 3$.
[b] QL: limit of quantitation based on $S/N \geqq 10$.

the recovery data of each fatty acid standard added to heated soybean oil. A high recovery of 94.4–102.7% was attained for all the 14 fatty acid standards, which was higher than a previous study by Indarti, Majid, Hashim, and Chong (2005), who analyzed the fatty acid content in fish oil and reported the recovery to be approximately 80%. This difference may be due to variation in extraction

Table 3
Recovery data of fatty acid methyl esters standards when added to heated soybean oil

Fatty acids	Recovery (%)						Total average
	5000 ppm			10,000 ppm			
	First	Second	Average	First	Second	Average	
C14:0	100.1	102.9	101.5	103.1	104.4	103.8	102.7 ± 1.6
C16:0	96.1	97.0	96.6	97.2	98.3	97.8	97.2 ± 0.9
C16:1,9*t*	94.0	96.2	95.1	95.2	97.6	96.4	95.8 ± 0.9
C16:1,9*c*	96.1	97.4	96.8	97.1	98.0	97.6	97.2 ± 0.6
C18:0	97.2	98.5	97.9	97.7	99.1	98.4	98.2 ± 0.4
C18:1,6*t*	94.8	96.3	95.5	96.3	98.3	97.3	96.4 ± 1.3
C18:1,9*t*	95.5	94.3	94.9	94.8	96.8	95.8	95.4 ± 0.7
C18:1,11*t*	93.2	94.6	93.9	95.7	97.2	96.5	95.2 ± 1.8
C18:1,9*c*	96.1	97.5	96.8	96.7	98.6	97.7	97.2 ± 0.6
C18:2,9*t*12*t*	94.9	96.8	95.8	96.0	98.4	97.2	96.5 ± 1.0
C18:2,9*c*12*c*	93.7	95.8	94.7	96.3	97.1	96.7	95.7 ± 1.4
C18:3,9*t*12*t*15*t*	95.4	93.3	94.3	95.2	96.4	95.8	95.0 ± 1.0
C20:0	92.3	93.1	92.7	96.5	95.7	96.1	94.4 ± 2.4
C18:3,9*c*12*c*15*c*	95.9	97.1	96.5	96.4	98.4	97.4	96.9 ± 0.6

procedure. It is also possible that the methyl esterification method (the boron-trifluoride method) used in our experiment may enhance the recovery substantially. Lee, Wang, and Ming (1990) compared the effect of four methyl esterification methods on the recovery of fatty acids in salad oil and depicted that a high recovery (>96%) could be achieved by either boron-trifluoride, sulfuric acid-reflux or tetramethyl-ammonium-salt method. Conversely, a low recovery (85.7%) was yielded by the sodium methoxide method, probably because of moisture absorption and decomposition into sodium hydroxide, resulting in an incomplete esterification.

3.2. Fatty acid composition change in soybean oil during heating

Tables 4–9 show the fatty acid composition in unhydrogenated and hydrogenated soybean oil. Fresh unhydrogenated soybean oil was found to contain three saturated fatty acids (C16:0, C18:0 and C20:0), 3 *cis* unsaturated fatty acids (C18:1, C18:2 and C18:3), of which linoleic acid constituted the largest portion (407.8 mg/g), followed by oleic acid (198.9 mg/g). However, in fresh hydrogenated soybean oil, three saturated fatty acids (C16:0, C18:0 and C20:0), 2 *cis* unsaturated fatty acids (C18:1 and C18:2) and four *trans* fatty acids (C18:1, Δ 6*t*; C18:1, Δ9*t*; C18:1, Δ11*t* and C18:2, Δ9*t*Δ12*t*) were present, with *cis* oleic acid (199.5 mg/g) dominating, followed by *trans* oleic acid (194.7 mg/g). No linolenic acid (C18:3) was detected, mainly because of conversion into linoleic acid (C18:2) or oleic acid (C18:1) or stearic acid (C18:0) during hydrogenation. By comparing the various *trans* forms of fatty acids, oleic acid was the most susceptible to formation in hydrogenated soybean oil. Karabulut, Kayahan, and Yaprak (2003) and Schmidt (2000) studied the formation of *trans* fatty acids during oil hydrogenation and the contents of *trans* forms of both oleic acid and linoleic acid followed an increased trend for the increase of reaction time.

3.2.1. Unhydrogenated soybean oil

Table 4 shows the fatty acid composition change of unhydrogenated soybean oil during heating at 160 °C for 4, 8, 12, 16, 20 and 24 h. Compared to fresh soybean oil, the levels of five fatty acids, namely, C16:0, C18:0, C18:1 (Δ9*c*), C18:2 (Δ9*c*Δ12*c*) and C18:3 (Δ9*c*Δ12*c*Δ15*c*) decreased along with increasing heating time, probably because of degradation during extensive heating. After prolonged heating for 24 h, a sharp decline by 20.2 (22.2%), 6.9 (22.9%), 46.6 (23.4%), 83.6 (25.5%) and 7.3 mg/g (20.4%) occurred for C16:0, C18:0, C18:1 (Δ9*c*), C18:2 (Δ9*c*Δ12*c*) and C18:3(Δ9*c*Δ12*c*Δ15*c*), respectively. However, no *trans* fatty acid was formed under this heating condition. The total amounts of fatty acids for 24 h samples were not the same as control samples, which could be accounted for by the instability of *cis* fatty acid under drastic condition for the former (Chen et al., 2001). In a study dealing with oxidative stability of methyl linoleate, methyl oleate and methyl stearate during heating, Chen et al. (2001) reported that the degradation could proceed faster than the peroxide formation at elevated temperature (200 °C). Table 5 shows the fatty acid composition change in unhydrogenated soybean oil during heating at 180 °C. Likewise, the contents of all the fatty acids exhibited a decreased tendency for the increase in heating time. After 24-h heating, a marked decline of 21.8 (24.0%), 10.6 (35.2%), 51.9 (26.1%), 11.7 (27.4%), 3.8 (35.0%) and 13.8 mg/g (38.7%) was observed for C16:0, C18:0, C18:1 (Δ9*c*), C18:2 (Δ9*c*Δ12*c*), C20:0 and C18:3 (Δ9*c*Δ12*c*Δ15*c*), respectively. Also, no *trans* fatty acid was formed in soybean oil heated at 180 °C. Similarly, the levels of all the fatty acids dropped pronouncedly during heating of soybean oil at 200 °C (Table 6), and a greater loss by 24.3 (26.7%), 12.0 (39.9%), 85.2 (42.8%), 144.1 (35.3%), 4.3 (39.8%) and 19.7 mg/g (55.2%) occurred for C16:0, C18:0, C18:1 (Δ9*c*), C18:2 (Δ9*c*Δ12*c*), C20:0 and C18:3 (Δ9*c*Δ12*c*Δ15*c*), respectively,after extensive heating for 24 h. Again, no

Table 4
Fatty acid composition change during heating of soybean oil at 160 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND[B]	ND	ND	ND	ND	ND	ND
C16:0	91.0 ± 1.9[a]	77.9 ± 1.1[bc]	75.7 ± 1.4[cd]	74.1 ± 1.0[de]	73.2 ± 1.5[de]	71.8 ± 1.0[e]	70.8 ± 1.5[e]
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	30.1 ± 1.5[a]	27.0 ± 0.8[bc]	26.2 ± 0.8[bcd]	25.6 ± 1.2[cde]	24.9 ± 0.2[cde]	24.1 ± 0.4[de]	23.2 ± 1.1[e]
C18:1,6*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,11*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*c*	198.9 ± 2.0[a]	173.5 ± 1.6[b]	170.6 ± 1.3[b]	164.6 ± 1.6[c]	160.6 ± 1.3[cd]	157.4 ± 1.1[d]	152.3 ± 3.1[e]
C18:2,9*t*12*t*	ND	ND	ND	ND	ND	ND	ND
C18:2,9*c*12*c*	407.8 ± 1.8[a]	357.1 ± 3.1[b]	352.5 ± 1.6[b]	345.0 ± 1.7[c]	340.6 ± 1.8[cd]	336.9 ± 1.8[d]	324.2 ± 2.0[e]
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	10.8 ± 0.9[a]	10.1 ± 1.5[ab]	9.6 ± 0.7[ab]	9.1 ± 0.7[ab]	8.7 ± 0.4[ab]	8.6 ± 0.1[b]	8.1 ± 1.0[b]
C18:3,9*c*12*c*15*c*	35.7 ± 1.0[a]	33.3 ± 0.6[bc]	32.1 ± 0.9[bc]	31.1 ± 1.2[cd]	29.3 ± 0.8[de]	28.4 ± 0.6[e]	28.4 ± 1.2[e]
Others	17.4	13.9	13.5	13.1	12.6	12.2	11.3
Subtotal (*trans*)	ND	ND	ND	ND	ND	ND	ND
Subtotal (*cis*)	642.4	563.8	555.1	540.7	530.4	522.7	504.8
Subtotal (sat)[D]	131.9	115.0	111.5	108.8	106.7	104.5	102.1
trans/*cis*	nil	nil	nil	nil	nil	nil	nil

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–e] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

Table 5
Fatty acid composition change during heating of soybean oil at 180 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND[B]	ND	ND	ND	ND	ND	ND
C16:0	91.0 ± 1.9[a]	73.2 ± 2.9[b]	71.4 ± 2.8[b]	71.5 ± 1.6[b]	71.3 ± 1.1[b]	70.5 ± 1.6[b]	69.2 ± 1.3[b]
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	30.1 ± 1.5[a]	23.4 ± 1.4[b]	22.0 ± 1.1[bc]	23.1 ± 1.5[b]	21.5 ± 0.8[bc]	21.0 ± 1.7[bc]	19.5 ± 1.3[bc]
C18:1,6*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,11*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*c*	198.9 ± 2.0[a]	169.8 ± 1.7[b]	167.9 ± 2.3[bc]	163.5 ± 2.0[cd]	159.2 ± 3.3[d]	151.7 ± 1.0[e]	147.0 ± 2.3[e]
C18:2,9*t*12*t*	ND	ND	ND	ND	ND	ND	ND
C18:2,9*c*12*c*	407.8 ± 1.8[a]	342.7 ± 3.6[b]	340.2 ± 2.2[bc]	334.6 ± 2.0[c]	324.8 ± 4.9[d]	305.8 ± 4.0[e]	296.1 ± 2.2[f]
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	10.8 ± 0.9[a]	8.3 ± 1.3[b]	9.3 ± 1.3[ab]	8.4 ± 1.5[ab]	7.6 ± 0.2[b]	7.5 ± 1.1[b]	7.0 ± 0.8[b]
C18:3,9*c*12*c*15*c*	35.7 ± 1.0[a]	29.0 ± 2.1[b]	27.4 ± 1.3[bc]	24.9 ± 1.3[cd]	23.3 ± 1.1[d]	22.6 ± 1.3[d]	21.9 ± 2.2[d]
Others	17.4	13.2	12.9	12.4	11.3	10.3	9.5
Subtotal (*trans*)	ND	ND	ND	ND	ND	ND	ND
Subtotal (*cis*)	642.4	541.5	535.5	523.0	507.3	480.0	464.9
Subtotal (sat)[D]	131.9	105.0	102.7	102.9	100.5	99.0	95.6
trans/*cis*	nil	nil	nil	nil	nil	nil	nil

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–f] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

trans fatty acid was formed in heated soybean oil at 200 °C. This result demonstrated that the higher the temperature, the faster the degradation of *cis* fatty acids (Frankel, 1998). Moreover, a drastic heating condition (>200 °C and >24 h) should be required to generate *trans* fatty acid formation in the oil. Theoretically, *cis* fatty acid should be more susceptible to heat loss than *trans* fatty acid (Frankel, 1998). Our result did prove that *cis* fatty

Table 6
Fatty acid composition change during heating of soybean oil at 200 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND[B]	ND	ND	ND	ND	ND	ND
C16:0	91.0 ± 1.9^{a}	70.0 ± 1.2^{b}	70.2 ± 0.5^{b}	69.4 ± 1.6^{bc}	68.3 ± 0.1^{bc}	67.4 ± 0.8^{bc}	66.7 ± 0.6^{c}
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	30.1 ± 1.5^{a}	21.2 ± 1.6^{b}	21.7 ± 0.7^{b}	20.0 ± 1.3^{bc}	20.3 ± 1.1^{bc}	19.4 ± 1.3^{bc}	18.1 ± 0.8^{c}
C18:1,6*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,11*t*	ND	ND	ND	ND	ND	ND	ND
C18:1,9*c*	198.9 ± 2.0^{a}	167.4 ± 3.5^{b}	158.7 ± 2.8^{c}	150.0 ± 2.4^{d}	139.1 ± 1.8^{e}	126.0 ± 2.0^{f}	113.7 ± 2.1^{g}
C18:2,9*t*12*t*	ND	ND	ND	ND	ND	ND	ND
C18:2,9*c*12*c*	407.8 ± 1.8^{a}	340.4 ± 3.3^{b}	329.2 ± 0.3^{c}	315.9 ± 2.3^{d}	299.5 ± 2.7^{e}	280.5 ± 2.6^{f}	263.7 ± 2.3^{g}
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	10.8 ± 0.9^{a}	7.6 ± 1.0^{bc}	7.9 ± 0.5^{bc}	7.7 ± 0.6^{bc}	7.4 ± 0.4^{bc}	7.0 ± 0.4^{c}	6.5 ± 0.5^{c}
C18:3,9*c*12*c*15*c*	35.7 ± 1.0^{a}	27.5 ± 2.1^{b}	26.8 ± 0.0^{b}	23.3 ± 1.1^{c}	21.1 ± 1.9^{cd}	18.4 ± 1.1^{de}	16.0 ± 1.0^{e}
Others	17.4	12.9	11.8	10.2	9.3	8.1	7.2
Subtotal (*trans*)	ND	ND	ND	ND	ND	ND	ND
Subtotal (*cis*)	642.4	535.2	514.6	489.2	459.6	424.9	393.4
Subtotal (sat)[D]	131.9	98.8	99.8	97.1	96.1	93.7	91.3
trans/*cis*	nil	nil	nil	nil	nil	nil	nil

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–g] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

Table 7
Fatty acid composition change during heating of hydrogenated soybean oil at 160 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND[B]	ND	ND	ND	ND	ND	ND
C16:0	106.0 ± 0.8^{a}	97.8 ± 0.8^{b}	95.3 ± 0.8^{c}	94.2 ± 0.8^{cd}	93.7 ± 0.6^{cd}	92.7 ± 0.6^{de}	91.0 ± 0.9^{e}
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	81.0 ± 0.8^{a}	77.2 ± 1.0^{b}	74.6 ± 1.1^{c}	73.7 ± 1.0^{cd}	72.6 ± 0.8^{cde}	71.6 ± 1.0^{de}	70.9 ± 0.8^{e}
C18:1,6*t*	50.5 ± 1.0^{a}	48.0 ± 1.1^{ab}	46.7 ± 1.4^{b}	46.0 ± 1.2^{bc}	45.7 ± 0.8^{bc}	43.5 ± 1.3^{cd}	42.1 ± 1.0^{d}
C18:1,9*t*	70.4 ± 1.1^{a}	66.5 ± 1.0^{b}	65.0 ± 1.0^{bc}	63.2 ± 0.6^{cd}	61.5 ± 1.0^{d}	58.9 ± 1.1^{e}	56.1 ± 1.2^{f}
C18:1,11*t*	73.8 ± 0.9^{a}	68.4 ± 0.7^{bc}	66.7 ± 1.0^{c}	64.2 ± 0.9^{d}	62.7 ± 1.1^{de}	60.4 ± 1.1^{e}	57.4 ± 1.3^{f}
C18:1,9*c*	199.5 ± 1.1^{a}	186.5 ± 1.1^{b}	182.9 ± 0.8^{c}	180.0 ± 1.6^{c}	174.9 ± 1.1^{d}	166.8 ± 1.4^{e}	158.6 ± 1.1^{f}
C18:2,9*t*12*t*	9.5 ± 0.5^{a}	9.1 ± 0.5^{ab}	8.8 ± 0.4^{abc}	8.7 ± 0.4^{abc}	8.3 ± 0.4^{bc}	8.2 ± 0.4^{bc}	7.8 ± 0.6^{c}
C18:2,9*c*12*c*	8.0 ± 0.5^{a}	8.0 ± 0.4^{a}	7.6 ± 0.6^{ab}	7.3 ± 0.6^{ab}	6.4 ± 0.6^{bc}	6.0 ± 0.4^{c}	5.6 ± 0.5^{c}
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	6.2 ± 0.3^{a}	6.1 ± 0.3^{a}	6.1 ± 0.4^{a}	5.9 ± 0.6^{a}	6.0 ± 0.4^{a}	6.1 ± 0.6^{a}	5.9 ± 0.4^{a}
C18:3,9*c*12*c*15*c*	ND	ND	ND	ND	ND	ND	ND
Others	80.7	72.3	70.1	68.9	67.8	66.1	64.4
Subtotal (*trans*)	204.2	192.0	187.2	182.0	178.1	171.0	163.4
Subtotal (*cis*)	207.5	194.4	190.5	187.3	181.3	172.8	164.2
Subtotal (sat)[D]	193.3	181.1	175.9	173.8	172.3	170.4	167.7
trans/*cis*	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–f] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

acid could undergo degradation under severe heating conditions, and the degraded products could be aldehyde, alcohol, ketone or hydrocarbon compounds, depending on heating temperature and time (Frankel, 1998). In a similar study dealing with heating of sunflower oil at 220, 240 and 270 °C for 5 h alone, Kamel and Kakuda (1994) reported no *trans* fatty acid formation at 220 °C. However, at 240 and 270 °C, the levels of *trans* fatty acids

Table 8
Fatty acid composition change during heating of hydrogenated soybean oil at 180 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND [B]	ND	ND	ND	ND	ND	ND
C16:0	106.0 ± 0.8^{a}	95.5 ± 1.1^{b}	94.4 ± 1.1^{b}	93.2 ± 1.1^{bc}	91.7 ± 0.8^{cd}	89.9 ± 0.6^{de}	87.7 ± 0.8^{e}
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	81.0 ± 0.8^{a}	74.9 ± 1.1^{bc}	73.6 ± 0.9^{cd}	73.1 ± 1.0^{cd}	71.7 ± 0.8^{de}	70.1 ± 1.0^{ef}	68.9 ± 1.1^{f}
C18:1,6*t*	50.5 ± 1.0^{a}	46.7 ± 1.3^{b}	46.2 ± 1.3^{bc}	45.3 ± 1.2^{bc}	43.3 ± 1.6^{cd}	41.5 ± 1.5^{de}	38.7 ± 1.6^{e}
C18:1,9*t*	70.4 ± 1.1^{a}	63.7 ± 1.3^{bc}	62.2 ± 1.3^{c}	61.3 ± 1.2^{c}	58.6 ± 1.2^{d}	55.4 ± 1.0^{e}	50.7 ± 0.6^{f}
C18:1,11*t*	73.8 ± 0.9^{a}	65.2 ± 1.6^{bc}	63.7 ± 1.6^{c}	63.7 ± 1.6^{c}	60.4 ± 1.1^{d}	57.4 ± 1.0^{e}	51.5 ± 0.8^{f}
C18:1,9*c*	199.5 ± 1.1^{a}	183.3 ± 1.3^{bc}	181.6 ± 1.1^{c}	177.7 ± 1.1^{d}	169.3 ± 1.2^{e}	159.1 ± 1.8^{f}	144.0 ± 2.4^{g}
C18:2,9*t*12*t*	9.5 ± 0.5^{a}	8.9 ± 0.5^{ab}	8.9 ± 0.5^{ab}	8.8 ± 0.4^{ab}	8.6 ± 0.5^{abc}	7.8 ± 0.6^{bc}	7.5 ± 0.4^{c}
C18:2,9*c*12*c*	8.0 ± 0.5^{a}	7.8 ± 0.5^{ab}	7.4 ± 0.5^{ab}	7.1 ± 0.6^{ab}	6.6 ± 0.5^{bc}	5.7 ± 0.4^{c}	5.5 ± 0.6^{c}
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	6.2 ± 0.3^{a}	5.9 ± 0.6^{a}	5.9 ± 0.5^{a}	6.1 ± 0.4^{a}	6.0 ± 0.4^{a}	5.8 ± 0.1^{a}	5.8 ± 0.5^{a}
C18:3,9*c*12*c*15*c*	ND	ND	ND	ND	ND	ND	ND
Others	80.7	70.1	69.1	67.5	65.2	63.0	60.4
Subtotal (*trans*)	204.2	184.5	180.9	179.0	170.8	162.1	148.2
Subtotal (*cis*)	207.5	191.0	189.0	184.7	175.8	164.7	149.5
Subtotal (sat)[D]	193.3	176.2	173.8	172.4	169.4	165.7	162.4
trans/*cis*	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–g] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

Table 9
Fatty acid composition change during heating of hydrogenated soybean oil at 200 °C for varied length of time

Fatty acid (mg/g)[C]	Heating (h)						
	Control[A]	4	8	12	16	20	24
C14:0	ND[B]	ND	ND	ND	ND	ND	ND
C16:0	106.0 ± 0.8^{3a}	93.8 ± 0.5^{b}	93.0 ± 1.3^{bc}	91.6 ± 0.9^{bc}	90.9 ± 1.3^{c}	87.8 ± 1.0^{d}	84.1 ± 1.2^{e}
C16:1,9*t*	ND	ND	ND	ND	ND	ND	ND
C16:1,9*c*	ND	ND	ND	ND	ND	ND	ND
C18:0	81.0 ± 0.8^{a}	72.6 ± 0.7^{bc}	71.6 ± 1.1^{cd}	73.1 ± 0.9^{bc}	69.7 ± 0.8^{de}	68.0 ± 0.8^{ef}	66.1 ± 1.1^{f}
C18:1,6*t*	50.5 ± 1.0^{a}	43.7 ± 1.7^{b}	44.6 ± 1.4^{b}	44.8 ± 1.0^{b}	40.4 ± 1.1^{c}	37.2 ± 1.5^{d}	35.2 ± 1.3^{d}
C18:1,9*t*	70.4 ± 1.1^{a}	61.7 ± 1.2^{bc}	59.0 ± 1.9^{cd}	59.0 ± 1.9^{cd}	56.3 ± 2.1^{d}	52.2 ± 1.6^{e}	45.8 ± 1.4^{f}
C18:1,11*t*	73.8 ± 0.9^{a}	63.3 ± 1.1^{b}	63.0 ± 1.2^{b}	61.2 ± 1.5^{b}	56.9 ± 1.3^{c}	52.7 ± 0.8^{d}	46.8 ± 1.3^{e}
C18:1,9*c*	199.5 ± 1.1^{a}	181.4 ± 1.1^{bc}	179.3 ± 1.3^{c}	172.4 ± 1.6^{d}	154.7 ± 1.2^{e}	143.9 ± 1.7^{f}	130.7 ± 1.6^{g}
C18:2,9*t*12*t*	9.5 ± 0.5^{a}	8.7 ± 0.1^{abc}	8.6 ± 0.4^{abc}	8.5 ± 0.6^{abc}	8.2 ± 0.6^{bcd}	7.6 ± 0.5^{cd}	7.2 ± 0.5^{d}
C18:2,9*c*12*c*	8.0 ± 0.5^{a}	7.2 ± 0.6^{ab}	6.7 ± 0.6^{abc}	6.4 ± 0.4^{bc}	6.2 ± 0.8^{bc}	5.6 ± 0.4^{c}	5.2 ± 0.4^{c}
C18:3,9*t*12*t*15*t*	ND	ND	ND	ND	ND	ND	ND
C20:0	6.2 ± 0.3^{a}	5.9 ± 0.5^{a}	5.9 ± 0.3^{a}	5.9 ± 0.3^{a}	5.7 ± 0.6^{a}	5.5 ± 0.4^{a}	5.7 ± 0.2^{a}
C18:3,9*c*12*c*15*c*	ND	ND	ND	ND	ND	ND	ND
Others	80.7	67.8	67.5	65.0	64.3	60.2	55.6
Subtotal (*trans*)	204.2	177.4	175.2	173.6	161.8	149.6	134.9
Subtotal (*cis*)	207.5	188.6	186.0	178.8	160.8	149.5	135.9
Subtotal (sat)[D]	193.3	172.2	170.4	170.5	166.3	161.3	155.8
trans/*cis*	1.0	0.9	0.9	1.0	1.0	1.0	1.0

[A] Control: fresh unhydrogenated soybean oil.
[B] ND: not detected.
[C] Means of duplicate analyses ± standard deviation.
[D] Sat: saturated fatty acid.
[a–g] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).

rose by 3% and 11%, respectively. Also, no *trans* fatty acid formation was observed in several vegetable oils when heated at 170 and 350 °C for 30 min or 200 and 220 °C for 16 h, and thus Möllenken (1998) concluded that *trans* fatty acids would be difficult to form unless a severe cooking condition was used. This phenomenon further proved that the heating conditions in our experiment are inadequate to induce formation of *trans* fatty acids.

3.2.2. Hydrogenated soybean oil

Table 7 shows the fatty acid composition change in hydrogenated soybean oil during heating at 160 °C for 4, 8, 12, 16, 20 and 24 h. A loss of 8.2, 3.8, 3.9, 5.4 and 13.0 mg/g was reached 4 h after heating for C16:0, C18:0, C18:1 (Δ9*t*), C18:1 (Δ11*t*) and C18:1 (Δ9*c*), respectively. In comparison with fresh hydrogenated soybean oil, a distinct decrease by 15.0 (14.2%), 10.1 (12.5%), 8.4 (16.6%), 14.3 (20.3%), 16.4 (22.2%), 40.9 (20.5%), 1.7 (17.9%) and 2.4 mg/g (30.0%) was shown for C16:0, C18:0, C18:1 (Δ6*t*), C18:1 (Δ9*t*), C18:1 (Δ 11*t*), C18:1 (Δ9*c*), C18:2 (Δ9*t*Δ 12*t*) and C18:2 (Δ9*c*Δ12*c*), respectively, after 24-h heating. Likewise, both *cis* and *trans* fatty acids can undergo degradation simultaneously after extensive heating. Nevertheless, no *trans* fatty acid was formed under this condition. A similar trend was observed for the fatty acid composition change during heating of hydrogenated soybean oil at 180 °C (Table 8). A large decline by 18.3 (17.3%), 12.1 (15.0%), 11.8 (23.4%), 19.7 (28.0%), 22.3 (30.2%), 55.5 (27.8%), 2.0 (21.1%) and 2.5 mg/g (31.3%) was attained 24 h after heating for C16:0, C18:0, C18:1 (Δ6*t*), C18:1 (Δ9*t*), C18:1 (Δ11*t*), C18:1 (Δ9*c*), C18:2 (Δ9*t*Δ 12*t*) and C18:2 (Δ9*c*Δ12*c*), respectively. Also, no *trans* fatty acid was formed. The same tendency also applied to hydrogenated soybean oil when heated alone at 200 °C for 24 h (Table 9), i.e., the contents of C16:0, C18:0, C18:1 (Δ6*t*), C18:1 (Δ9*t*), C18:1 (Δ11*t*), C18:1 (Δ9*c*), C18:2 (Δ9*t*Δ12*t*) and C18:2 (Δ9*c*Δ12*c*) showed a greater decrease by 21.9 (20.7%), 14.9 (18.4%), 15.3 (30.3%), 24.6 (34.9%), 27.0 (36.6%), 68.8 (34.5%), 2.3 (24.2%) and 2.8 mg/g (35.0%), respectively, while no *trans* fatty acid formation was observed.

By comparison of the results shown above, it may be concluded that an Agilent HP-88 column could provide effective separation of eight *trans* fatty acids and nine *cis* fatty acids within 31 min. Both the degradation of *cis* and *trans* fatty acids could proceed fast at elevated temperature. No *trans* fatty acid was formed in unhydrogenated and hydrogenated soybean oil during heating at 160, 180 or 200 °C for 24 h, implying that *trans* fatty acid can only be formed under drastic heating condition. The technique developed in this study may be adopted as a reference method for routine analysis of *trans* fatty acids in commercial food products. As mentioned before, the nutrition labeling of *trans* fatty acids has become an urgent issue to solve, and application of this method can provide valuable information to assist consumers in maintaining healthy dietary practices. Further research is necessary to study the formation of *trans* fatty acids in bakery and fried products with hydrogenated oil as heating medium.

References

Adlof, R. O. (1994). Separation of *cis* and *trans* unsaturated fatty acid methyl esters by silver ion high performance liquid chromatography. *Journal of Chromatography, 659*, 95–99.

Adlof, R. O., Copes, L. C., & Emken, E. A. (1995). Analysis of the monoenoic fatty acid distribution in hydrogenated vegetable oils by silver-ion high performance liquid chromatography. *Journal of American Oil Chemists' Society, 72*, 571–574.

American Oil Chemists' Society. (1990). *Official Methods and Recommended Practices, Method Ce 1c-89*, Champaign, Illinois.

Aro, A., Amaral, E., Kesteloot, H., Rimestad, A., Thamm, M., & Poppel, G. (1998). *Trans* fatty acids in French fries, soups, and snacks from 14 European countries: the transfair study. *Journal of Food Composition and Analysis, 11*, 170–177.

Chen, J. F., Tai, C. Y., Chen, Y. C., & Chen, B. H. (2001). Effects of conjugated linoleic acid on the oxidation stability of model lipids during heating and illumination. *Food Chemistry, 72*, 199–206.

Christie, W. W., & Breckenridge, G. H. M. (1989). Separation of *cis* and *trans* isomers of unsaturated fatty acids by high performance liquid chromatography in the silver ion mode. *Journal of Chromatography, 469*, 261–269.

Food and Drug Administration (2003). Food labeling: *trans* fatty acids in nutrition labeling, nutrient content claims, and health claims. *Federal Register, 68*(133), 41434–41506.

Frankel, E. N. (1998). *Lipid oxidation. P55-77*. Dundee, Scotland: The Oily Press Ltd.

Han, S. N., Leka, L. S., Lichtenstein, A. H., Ausman, L. M., Schaefer, E. J., & Meydani, S. N. (2002). Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *Journal of Lipid Research, 43*, 445–452.

Indarti, E., Majid, M. I. A., Hashim, R., & Chong, A. (2005). Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. *Journal of Food Composition and Analysis, 18*, 161–170.

International Conference on Harmonization (ICH). (1996). *Guideline on the validation of analytical procedures: Methodology Q2B*, November.

Juanéda, P. (2002). Utilization of reversed-phase high performance liquid chromatography as an alternative to silver-ion chromatography for the separation of *cis* and *trans* C18:1 fatty acid isomers. *Journal of Chromatography, 954*, 285–289.

Kamel, B., & Kakuda, Y. (1994). *Technological Advances in Improved and Alternative Sources of Lipids*. UK: Chapman & Hall (pp. 296–303).

Karabulut, I., Kayahan, M., & Yaprak, S. (2003). Determination of changes in some physical and chemical properties of soybean oil during hydrogenation. *Food Chemistry, 81*, 453–456.

Kris-Etherton, P. M. (1995). *Trans* fatty acids and coronary heart disease risk. *American Journal of Clinical Nutrition, 62*, 655–708.

Kummerow, F. A., Zhou, Q., Mahfouz, M. M., Smiricky, M. R., Grieshop, C. M., & Schaeffer, D. J. (2004). *Trans* fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipids of arterial cells. *Life Science, 74*, 2707–2723.

Lee, M. H., Wang, M. L., & Ming, P. U. (1990). Effects of methyl esterification method on analysis of fatty acid. *Food Science, 17*, 1–10 (in Chinese).

Libbey, P., Rid, K., & Maseri, P. M. (2002). Inflammation and atherosclerosis. *Circulation, 105*, 1135–1143.

Mensink, R. P., & Katan, M. B. (1990). Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine, 323*, 439–445.

Mensink, R. P., & Katan, M. B. (1993). *Trans* monounsaturated fatty acids in nutrition and their impact on serum lipoprotein levels in man. *Progress in Lipid Research, 32*, 111–122.

Möllenken, H. (1998). *Trans* fatty acids in heated hemp seed oil. *Journal of International Hemp Association, 5*(1), 21–25.

Romero, A., Cuesta, C., & Sánchez-Muniz, F. J. (2000). *Trans* fatty acid production in deep fat frying of frozen foods with different oils and frying modalities. *Nutrition Research, 20*, 599–608.

Ruiz-Jimenez, J., Priego-Capote, F., & Luque de Castro, M. D. (2004). Identification and quantification of *trans* fatty acids in bakery products by gas chromatography–mass spectrometry after dynamic ultrasound-assisted extraction. *Journal of Chromatography A, 1045*, 203–210.

SAS. (2003). SAS Procedures and SAS/Graph User's Guide. Version 6; Cary, NC: SAS Institute, Inc.

Schmidt, S. (2000). Formation of *trans* unsaturation during partial catalytic hydrogenation. *European Journal of Lipid Science and Technology, 102*, 646–648.

Taubes, G. (2002). *Cardiovascular disease. Does inflammation cut to the heart of the matter?* Science.

Vicario, I. M., Griguol, V., & Leon-Camacho, M. (2003). Multivariate characterization of the fatty acid profile of Spanish cookies and bakery products. *Journal of Agricultural and Food Chemistry, 51*, 134–139.