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Determination of Thermally Induced *trans*-Fatty Acids in Soybean Oil by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy and Gas Chromatography Analysis

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ABSTRACT: The intake of edible oil containing *trans*-fatty acids has deleterious effects mainly on the cardiovascular system. Thermal processes such as refining and frying cause the formation of *trans*-fatty acids in edible oil. This study was conducted to investigate the possible formation of *trans*-fatty acids because of the heat treatment of soybean oil. The types of *trans*-fatty acids in heated soybean oil are determined by attenuated total reflectance Fourier transform infrared spectroscopy and gas chromatography−mass spectrometry methods. The effects of the heating temperature on the *trans*-fatty acids in soybean oil were evaluated using gas chromatography flame ionization detection analysis. Results show that heat treatment at 240 °C causes the formation of *trans*-fatty acids in soybean oil and the amount of *trans*-fatty acids increases with heating time. The only peak observed at 966 cm^{-1} of the samples indicates the formation of nonconjugated *trans* isomers in the heated soybean oil. The major types of *trans*-fatty acids formed were *trans*-polyunsaturated fatty acids. Significant increases ($P < 0.05$) in the amounts of two *trans*-linoleic acids (C18:2-9c,12t and C18:2-9t,12c) and four *trans*-linolenic acids (C18:3-9c,12c,15t, C18:3-9t,12c,15c, and C18:3-9t,12t,15c/C18:3-9t,12c,15t) in soybean oil heated to temperatures exceeding 200 °C were compared with those of the control sample. The heating temperature and duration should be considered to reduce the formation of *trans*-fatty acids during thermal treatment.

KEYWORDS: *trans-fatty acids, heat treatment, soybean oil, effect of temperature*

INTRODUCTION

Soybean oil, which constitutes approximately 30% of all oils and fats produced,[1] is one of the most consumed vegetable oils in the world. Given its high content of essential fatty acids such as linoleic and linolenic acid, soybean oil is considered nutritionally beneficial for human health. However, heat treatments such as frying induce the formation of diverse amounts of *trans*-fatty acids (TFAs) in soybean oils.[2,3] Epidemiologically, the intake of TFAs increases the risk of cardiovascular disease by increasing unhealthy low-density lipoproteins and lowering healthy high-density lipoproteins.[4] A 2% increase in TFAs reportedly increases the risk of cardiovascular disease by 23%.[5]

Many studies have reported that the major sources of TFAs for consumers are partially hydrogenated oils, but the accumulation of TFAs in edible oils by heating has also attracted increasing attention recently. The *trans* isomers in hydrogenated oil are mainly *trans*-C18:1 (C18:1-6t, C18:1-9t, and C18:1-11t), whereas the *trans* isomer compositions are significantly different in heated oil.[3] Previous studies conducted by Tsuzuki have proven that *cis*−*trans* isomerization during the heating of triglycerides occurs without double bond migration.[6] However, studies conducted by Destaillats showed that thermal treatment at 180 or 240 °C resulted in the formation of *cis*/*trans*-conjugated linoleic acid (CLA) isomers because of 1,3-sigmatropic rearrangement.[7] Although CLA is a type of TFA, it is not considered a "bad" *trans*-fat because of its many health benefits.[8] Therefore, the types of TFAs induced by the thermal treatment of soybean oil need to be verified.

The purpose of the present study is to determine the types of TFAs in heated soybean oil using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and gas chromatography−mass spectrometry (GC−MS) analysis, and the levels of each TFA in soybean oil heated to different temperatures are measured by gas chromatography flame ionization detection (GC-FID) analysis. Some literature has reported that antioxidants could possibly suppress the accumulation of *trans* isomers[6,9] and the isomerization of unsaturated fatty acids also depends on the presence or absence of oxygen.[2] In our study, no extra antioxidant was involved and the sample was heated with head space air to imitate the real situation of the oil frying process. The effect of different heating temperatures on the composition of fatty acids in soybean oil was also studied.

MATERIALS AND METHODS

Materials. Edible refined soybean oil was purchased from a local market (Beijing, China). The mix standards of methyl esters, including C18:1 isomers, C18:2 isomers, and C18:3 isomers, were purchased from Sigma-Aldrich (St. Louis, MO). Other standards of methyl esters were purchased from NU-CHEK Prep, Inc. (Elysian, MN). Undecanoic acid triglyceride used as an internal standard for GC-FID analysis was obtained from NU-CHEK Prep. Isooctane obtained from Fisher Scientific (Fair Lawn, NJ) was used as a chromatographic organic solvent. Silicone oil (viscosity 500 cSt) obtained from Dingye Industrial Co., Ltd. (Beijing, China) was used as the heating material.

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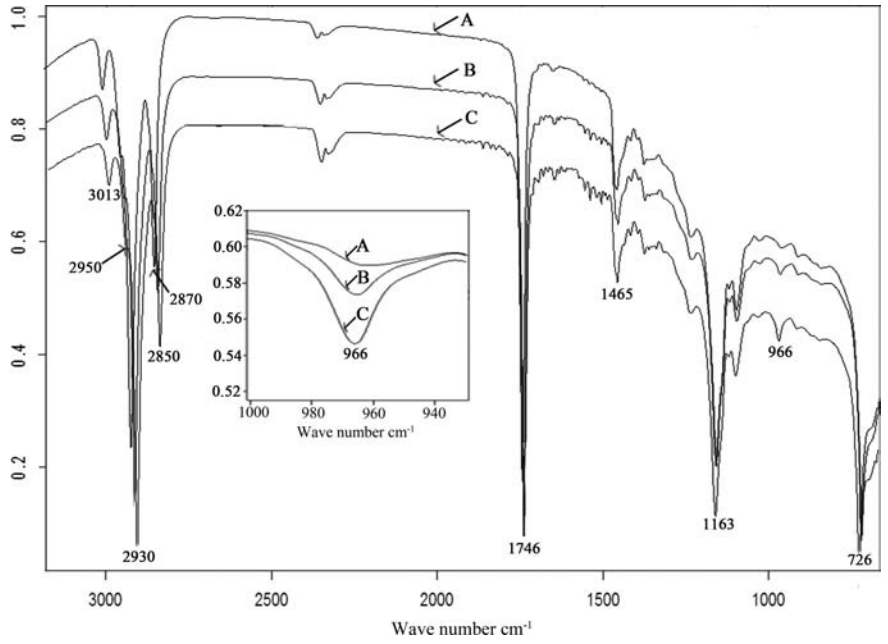

Figure 1. Infrared spectra of soybean oil unheated and heated (A, unheated; B, heated at 240 °C for 6 h; C, heated at 240 °C for 12 h).

Thermal Processing Procedures. Each 5 mL ampule bottle was filled with 3 g of soybean oil with air in the head space and then heated in a silicone oil bath. The maximum temperature was 240 °C and the maximum heating time was 12 h for each sample. The accuracy of temperature control during the oil heating procedures was ±2 °C.

Preparation of Fatty Acid Methyl Esters. Fatty acid methyl esters (FAMEs) were prepared according to the revised AOCS Official Method Ch 1-91.[10] A stoppered centrifuge vial was filled with 200 mg of the test sample. Then 2.0 mL of the internal standard was dissolved in isooctane followed by 0.1 mL of 2 mol/L methanolic KOH. The vial was shaken well for 30 s, and then the sample was centrifuged at 4000 rpm for 10 min. Then 20 μL of the supernatant layer was pipetted into a 1 mL volumetric flask, and the sample was diluted to the mark with isooctane.

ATR-FTIR Conditions. FTIR measurements were done on a Bruker Tensor 37 (Ettlingen, Germany) IR spectrometer operating under OPUS software in ATR mode. FTIR spectra were collected over the wavenumber range from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1} according to an industrial standard.[11] Air was used as the reference background material.

GC−MS Conditions. GC−MS analysis of FAMEs was performed on Shimadzu QP2010-Plus GC−MS instrument (Kyoto, Japan) coupled to a mass-selective detector. A CP-SIL 88 capillary column (100 m × 0.25 μm × 0.2 mm) was used for the separation of FAMEs. The initial temperature of 60 °C was maintained for 5 min, and then the temperature was increased to 160 °C at a rate of 25 °C/min and maintained at 160 °C for 5 min. Finally, the temperature was increased to 225 °C at a rate of 2 °C/min and maintained at 225 °C for 15 min.[12] The injection volume was 1 μL with a split ratio of 1:10, and helium (99.999%) was used as the carrier gas with a flow rate of 2.9 mL/min. The injector and interface temperatures were both 230 °C. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 30−400 *m*/*z*.

GC-FID Conditions. The prepared FAMEs were analyzed using a GC-2010 chromatograph (Shimadzu, Kyoto, Japan) equipped with a CP-SIL 88 fused silica capillary column (100 m × 0.25 μm × 0.2 mm) and a flame ionization detector. High-purity nitrogen (99.999%) was used as the carrier gas. The temperature program, injection volume, and mode were the same as the parameters for the GC−MS analysis. The injector and detector temperatures were 230 and 230 °C, respectively.

Calculations and Statistical Analysis. Peak identification of the fatty acids in the analyzed soybean oil samples was conducted by comparison with the retention times and mass spectra of known standards. Each sample was analyzed three times, and the data obtained were analyzed by the least-squares difference method ($P < 0.05$) using DPS software version 7.05 and were reported as the mean ± standard deviation.

RESULTS AND DISCUSSION

Analysis of TFAs in Heated Soybean Oil Using ATR-FTIR. The infrared spectra of the soybean oil heated at different temperatures are shown in Figure 1, and the main infrared band assignments for all the triglycerides in soybean oil are presented in Table 1. The common features of the wavenumbers between 3013 and 726 cm^{-1} show the absorption of the functional groups of triglycerides in soybean oil. The isomers containing *trans* configurations are expected to absorb at 966 cm^{-1} in the infrared spectrum because of the bending vibrations of *trans* =CH groups. As shown in the inside chart of Figure 1, the peak at 966 cm^{-1} increased with heating time at 240 °C. Therefore, the formation of *trans* isomers in the heated soybean oil was confirmed according to this infrared spectrum. Christy [13] demonstrated that CLAs were produced during the heating of trilinolein to 250 °C. If CLAs were formed in the heated sample, two specific absorption peaks at approximately 987 and

Table 1. Main Infrared Band Assignments of Triglycerides in Soybean Oil

frequency (cm^{-1})	functional group	mode of vibration
3013	=CH	stretching
2950	CH (CH_3)	asymmetric stretch
2930	CH ($-CH_2$)	asymmetric stretch
2870	CH (CH_3)	symmetric stretch
2850	CH ($-CH_2$)	symmetric stretch
1746	−C=O ester	Fermi resonance
1465	−CH ($-CH_2-$, CH_3)	bending
1163	−C−O	stretching
966	=CH *trans*	stretching
726	$(-CH_2-)_n$, $n > 4$	rocking

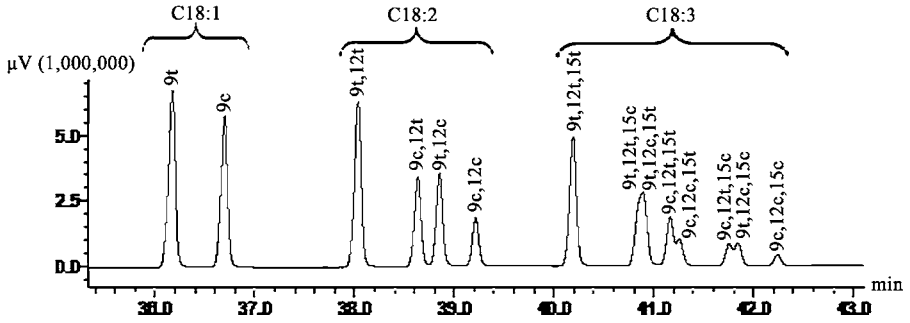

Figure 2. Chromatographic separation of the mixed standard of fatty acid methyl ester isomers separated by GC-MS using a CP-SIL column (100 m × 0.25 μm × 0.2 mm).

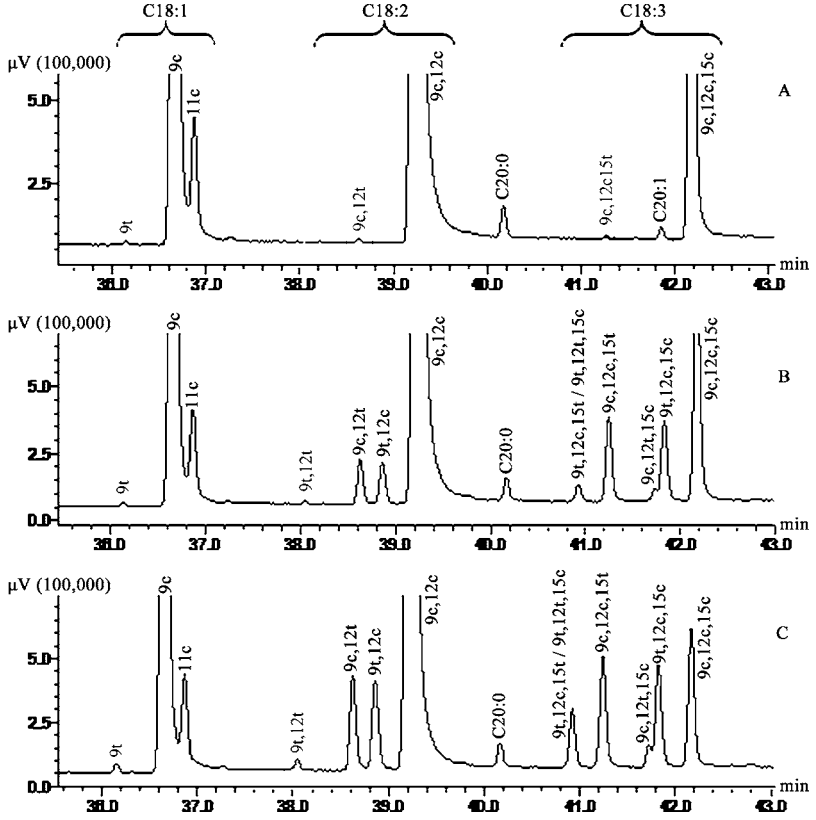

Figure 3. Chromatographic separation of fatty acid methyl ester isomers in soybean oil subjected to different heating treatments (A, unheated; B, heated at 240 °C for 6 h; C, heated at 240 °C for 12 h) and separated by GC-MS using a CP-SIL column (100 m × 0.25 μm × 0.2 mm).

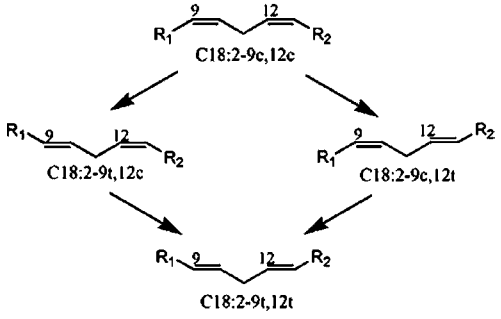

Figure 4. Isomerization tree of the formation of *trans*-C18:2 in heated soybean oil.

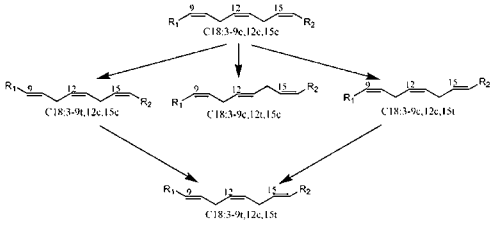

Figure 5. Isomerization tree of the formation of *trans*-C18:3 in heated soybean oil.

946 cm^{-1} were recorded from the conjugated *cis*/*trans* or *trans*/*cis* bond. However, no peaks were observed on both sides of the peak at 966 cm^{-1}. Thus, the *trans* isomers in soybean oil heated to 240 °C were nonconjugated *trans*-fatty acids.

Determination of TFAs in Heated Soybean Oil by GC–MS. The total ion chromatogram of the mix standards of oleic acid methyl ester isomers, linoleic acid methyl ester isomers, and linolenic acid methyl ester isomers are presented (Figure

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Table 2. Effect of the Heating Temperature on the Fatty Acid Composition in Soybean Oil (g/100 g of oil)[a]

fatty acid	unheated	160 °C	180 °C	200 °C	220 °C	240 °C
C14:0	0.065 ± 0.001 a	0.061 ± 0.002 a	0.060 ± 0.001 a	0.062 ± 0.004 a	0.063 ± 0.002 a	0.062 ± 0.001 a
C16:0	9.994 ± 0.166 ab	10.056 ± 0.115 a	9.812 ± 0.161 b	9.969 ± 0.165 ab	9.897 ± 0.109 ab	9.776 ± 0.050 b
C16:1	0.066 ± 0.001 a	0.062 ± 0.004 b	0.062 ± 0.001 b	0.059 ± 0.002 b	0.060 ± 0.001 b	0.059 ± 0.003 b
C18:0	4.051 ± 0.067 a	3.973 ± 0.096 ab	3.882 ± 0.079 b	3.954 ± 0.085 ab	3.978 ± 0.065 ab	3.899 ± 0.053 b
C18:1-9t	0.023 ± 0.002 de	0.027 ± 0.002 a	0.022 ± 0.001 abc	0.029 ± 0.002 ab	0.038 ± 0.001 bc	0.051 ± 0.003 c
C18:1-9c	19.461 ± 0.384 ab	19.655 ± 0.242 a	19.467 ± 0.342 ab	19.039 ± 0.369 bc	19.099 ± 0.297 bc	18.801 ± 0.141 c
C18:1-11c	1.323 ± 0.040 a	1.296 ± 0.048 ab	1.278 ± 0.028 ab	1.304 ± 0.037 ab	1.298 ± 0.038 ab	1.245 ± 0.012 b
C18:2-9t,12t	ND[b]	ND	ND	0.045 ± 0.006 b	0.054 ± 0.003 b	0.084 ± 0.005 a
C18:2-9c, 12t	0.037 ± 0.001 e	0.050 ± 0.003 de	0.060 ± 0.003 d	0.132 ± 0.002 c	0.412 ± 0.004 b	1.511 ± 0.028 a
C18:2-9t,12c	ND	ND	0.032 ± 0.002 d	0.107 ± 0.003 c	0.394 ± 0.007 b	1.571 ± 0.035 a
C18:2-9c, 12c	52.928 ± 1.018 a	52.669 ± 0.659 a	52.216 ± 0.825 ab	51.897 ± 0.917 ab	51.028 ± 0.765 b	47.016 ± 0.419 c
C20:0	0.311 ± 0.010 a	0.299 ± 0.012 b	0.291 ± 0.009 ab	0.297 ± 0.009 ab	0.301 ± 0.006 ab	0.293 ± 0.005 b
C18:3-9t,12t,15c/C18:3-9t,12c,15t	ND	ND	ND	ND	0.123 ± 0.004 b	0.910 ± 0.014 a
C18:3-9c,12c,15t	0.028 ± 0.002 e	0.036 ± 0.002 e	0.089 ± 0.004 d	0.354 ± 0.009 c	1.022 ± 0.015 b	1.784 ± 0.034 a
C18:3-9c,12t,15c	ND	0.006 ± 0.002 c	0.006 ± 0.002 c	0.012 ± 0.001 c	0.068 ± 0.001 b	0.210 ± 0.012 a
C20:1	0.162 ± 0.002 a	0.162 ± 0.002 a	0.162 ± 0.002 a	0.162 ± 0.002 a	0.162 ± 0.002 a	0.162 ± 0.002 a
C18:3-9t,12c,15c	ND	ND	0.058 ± 0.002 d	0.300 ± 0.009 c	0.932 ± 0.022 b	1.635 ± 0.025 a
C18:3-9c,12c,15c	8.763 ± 0.180 a	8.630 ± 0.141 a	8.225 ± 0.151 b	7.632 ± 0.165 c	5.789 ± 0.084 d	2.320 ± 0.026 e
C22:0	0.329 ± 0.027 a	0.307 ± 0.027 ab	0.309 ± 0.004 ab	0.313 ± 0.006 ab	0.295 ± 0.005 b	0.297 ± 0.005 b
C24:0	0.109 ± 0.007 a	0.104 ± 0.003 ab	0.099 ± 0.002 b	0.107 ± 0.005 ab	0.107 ± 0.007 ab	0.110 ± 0.006 a
∑TFAs	0.089	0.119	0.267	0.978	3.044	7.756
∑SFAs	14.859	14.799	14.453	14.702	14.641	14.437
∑*cis*-UFAs	82.702	82.473	81.409	80.094	77.436	69.603
∑total FAs	97.650	97.390	96.129	95.774	95.121	91.796

[a]Values are given as the means of triplicate analyses ± standard deviation. Symbols bearing different letters in the same row are significantly different ($P < 0.05$). [b]Not detected.

2). The peaks were identified according to the standards supplied with the chromatograph, and the retention time of each standard was obtained. The 11 *trans* isomers (see Figure 2) would be detected because they are probably formed through the isomerization of oleic acid, linoleic acid, and linolenic acid, respectively, during heat treatment. The peaks of oleic acid and linoleic acid methyl ester isomer were separated well and could be easily distinguished. However, the retention times of some *trans*-linolenic acid methyl esters such as C18:3-9t,12t,15c and C18:3-9t,12c,15t were very similar. The two compounds shared almost the same peak, so the amount of C18:3-9t,12t,15c and C18:3-9t,12c,15t was expressed as the sum of the two compounds.

Identification of each peak in the chromatograms (Figure 2) was based on their characteristic ion fragmentation spectra (e.g., C18:1, *m/z* 69, 83, 97, 111, 264, and 296; C18:2, *m/z* 67, 81, 95, 109, 262, and 294; C18:3, *m/z* 67, 79, 93, 108, 263, and 292). However, the *cis* and *trans* isomers were difficult to distinguish under EI-MS. Thus, the retention time of each standard was used to identify the isomers. On the basis of the chromatogram, C18:1-9c, C18:2-9c,12c, and C18:3-9c,12c,15c were detected as the main unsaturated fatty acids in the untreated soybean oil. Small amounts of C18:1-9t, C18:2-9c,12t, and C18:3-9c,12c,15t were also observed in the control sample. These *trans* fatty acids were probably produced during the oil refining process because of the high temperature. The heating during deodorization and physical refining causes the formation of TFAs.[14,15]

A range of *trans* isomers was detected in the thermally treated samples, and *trans*-linoleic and *trans*-linolenic acids were observed as the major *trans*-fatty acids in soybean oil heated at 240 °C. The amount of each TFA increased with heating time as shown in the graph. According to Figure 3, the *cis*−*trans* isomerization of C18:2-9c,12c induced the formation of C18:2-9c,12t, C18:2-9t,12c, and C18:2-9t,12t, whereas the *trans* isomers of C18:3-9c,12c,15c were C18:3-9t,12t,15c/C18:3-9t,12c,15t, C18:3-9c,12c,15t, C18:3-9c,12t,15c, and C18:3−9t,12c,15c. The isomerization of double bonds requires rotational energy.[16] Thus, the formation of isomers with two *trans* double bonds requires more energy than that of isomers with only one *trans* double bond. The isomerization of linoleic acid and linolenic acid based on the chromatograms is shown in Figures 4 and 5, respectively. All the TFAs were formed during the thermal process via an isomerization path without double bond migration.

Effect of the Heating Temperature on TFAs in Heated Soybean Oil. Soybean oil samples were heated at 160, 180, 200, 220, and 240 °C for 12 h, and all fatty acids in the examined samples were analyzed quantitatively and qualitatively under the GC-FID analysis conditions. The overlap of *trans*-linolenic acid and C20:1 had a minor effect on the quantitative analysis because the amount of C20:1 in heated oil was significantly lower than that of C18:3-9t,12c,15c and the amount of C20:1 was considered to be constant for approximate analysis.

An increase in the amount of total *trans*-fatty acids was observed with the increase of the heating temperature over a period of 12 h (Table 2). As presented by Choe and Min,[17] the oxidation, decomposition, and polymerization of oil are very common chemical reactions that lead to reduced amounts of fatty acids. The changes in unsaturated fatty acids, especially polyunsaturated fatty acid (PUFAs) such as C18:3-9c,12c,15c, were more significant ($P < 0.05$) than those in saturated fatty acid because the double bond had weak thermal stability.

A gradual increase in the amount of *trans*-C18:1 was observed compared with the rapid increase of *trans*-C18:2 and *trans*-C18:3. This result occurred probably because the height of the internal rotational barrier of the double bond in C18:1 is higher than that of the other two PUFAs. A heating temperature above 200 °C significantly ($P < 0.05$) promoted the formation of TFAs (Table 2). Similar results have been reported by Juanéda,[18] who investigated the influence of heat on linoleic acid isomers in sunflower oil. When the curve of the total TFA content was fit with the temperature between 160 and 240 °C, it showed an exponential increase with R^2 over 0.990. The total amount of TFAs in soybean oil heated at 220 °C for 12 h was 3.044 g/100 g of oil, which was over the *trans*-fat limit of 2 g/100 g fat in some countries such as Denmark,[19] and studies have shown that *trans*-C18:2 causes greater cell damage than *trans*-C18:1.[20] Therefore, the risk of TFA intake from soybean oil heated at high temperature is not negligible.

It has been reported that a molecule of *trans* configuration is more stable and the activation energy is so high that isomerization reaction should take place under thermal stress.[13] Our study confirms the thermal stability of the *trans* isomers compared with *cis* isomers. Further research should be conducted on the temperature and duration of the heat treatment during oil refining and frying to avoid the risk of disease caused by TFAs.

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Notes

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