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REVIEW

Study of the Formation of *trans* Fatty Acids in Model Oils (triacylglycerols) and Edible Oils during the Heating Process

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Abstract

Recent epidemiological studies have suggested the adverse effects of an excess intake of *trans* fatty acids on human health. This study investigates the relation between the formation of *trans* fatty acids and heating. The mechanism of heat-induced *cis/trans* isomerization was first resolved by using the model lipids (triacylglycerols). Then the formation of *trans* fatty acids in edible oil during heating and frying were examined to accurately evaluate the content of *trans* fatty acids found in fried or heated food.

When one kind of unsaturated triacylglycerol—triolein (9-*cis*, 18:1)—was heated at around 180°C, small amounts of isomerization products dependent on heating temperature and heating period were obtained. Heat-induced isomerization is strongly correlated with the thermal oxidation of double bonds. And several edible antioxidants such as δ-tocopherol, sesamol, and rosemary extract effectively suppressed heat-induced *trans* isomerization.

In comparison with triacylglycerols, many small formations in *trans* fatty acids were observed in commercially available several edible oils when heated at around 180°C. The antioxidants coexisting in each edible oil also help suppress the formation of heat-induced *trans* fatty acids.

A frying model system was then used to estimate increases in *trans* fatty acids during cooking. Sliced raw potatoes (100 g) were fried in commercially available corn oil at 180°C, and 30 frying cycles were performed. A small change in the content of *trans* fatty acids in the frying corn oil suggests that an ordinary frying process using unhydrogenated edible oils has little impact on the dietary intake of *trans* fatty acids.

Discipline: Food
Additional key words: double bonds, heat-induced, *trans*-Isomerization, unsaturated fatty acids

Introduction

Several pieces of epidemiologic evidence and case-control studies have revealed that an excess intake of *trans* fatty acids is associated with the risk of coronary heart disease.[4,7] Given the adverse effects on health caused by such excess intake, some countries have begun labeling the content of *trans* fatty acids in processed foods to increase consumer awareness about *trans* fatty acids. Such acids are generally defined as unsaturated fatty acids that contain non-conjugated carbon-carbon double bounds in a *trans* configuration. The *trans* fatty acids in foods are derived from the chemical hydrogenation of vegetable and fish oils, the refinement process of edible oils from crude oil, and microbial biohydrogenation in the digestive tract of ruminant animals.

Foods containing partially hydrogenated edible oils are major sources of *trans* fatty acids in the diet. Conversely, the consumption of *trans* fatty acids from refined edible oils is not particularly large. In order to assess the intake of *trans* fatty acids from edible oil, the *trans* fatty acids induced during cooking should be considered, however, in addition to those produced in such manufacturing processes as thermal refining, bleaching, and deodorization.[3] Recent studies have suggested that a small amount of *trans* fatty acids in edible oils is produced during the cooking and frying processes, and that such acids accumulate considerably only when these oils are subjected to such severe conditions as heating at temperatures higher than 250°C.[2,5] We had previously found

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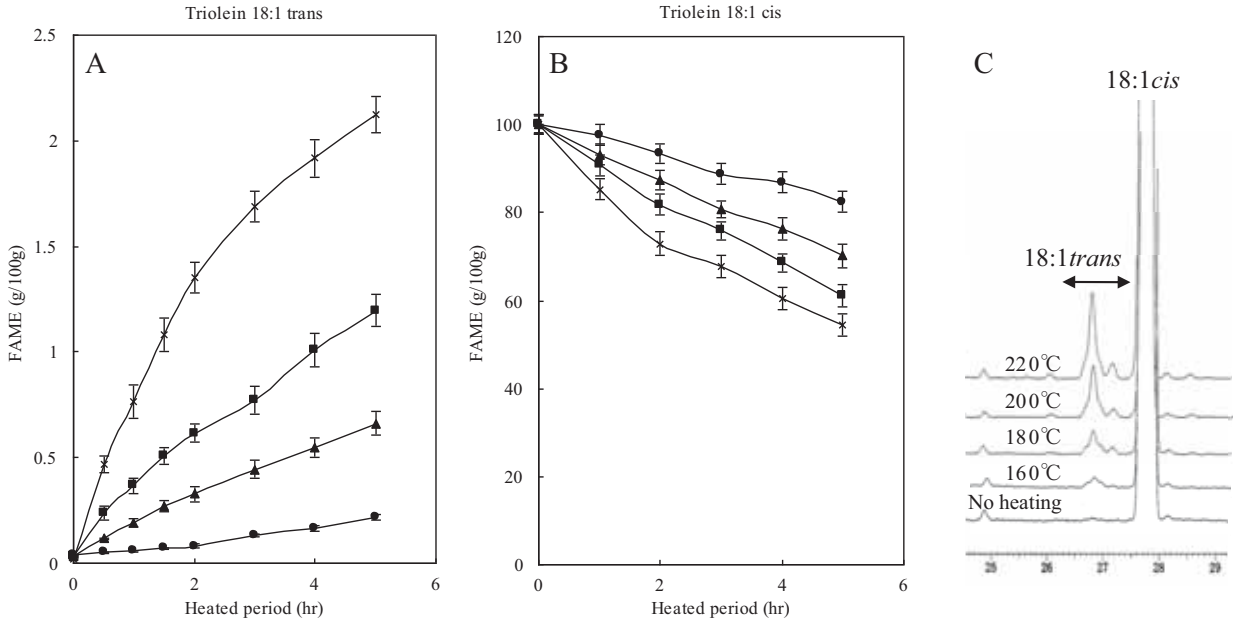

Fig. 1. Time course of *trans* 18:1 isomers formation (A) and *cis* 18:1 isomers degradation (B) in triolein heated at 160°C, 180°C, 200°C and 220°C

The *trans* 18:1 isomers were eluted between 26.5 and 27.4 minutes, and *cis* 18:1 isomers were eluted between 27.6 and 28.7 minutes by GC analysis (C). The absolute amount of each isomer was calculated from the peak intensity of internal standard (17:0) FAME. The values represent the means ± SD of three experiments.
—●— : 160C, —▲— : 180C, —■— : 200C, —×— : 220C.

that a small increase in the *trans* isomers of unsaturated triacylglycerols heated at around 180°C is correlated with thermal oxidation of the double bonds.[8] However, very little information is available about the relation between thermally induced lipid oxidation and the heat-induced isomerization of double bonds in the unsaturated fatty acids of edible oils and triacylglycerols.

In order to elucidate the mechanisms of the heat-induced *trans* isomerization of double bonds in lipids in the present study, we first used highly purified unsaturated triacylglycerols, as most commercially available edible oils contain more than one antioxidant that might have different effects on the thermal oxidation reaction and heat-induced *trans* isomerization. Several antioxidants were added to heating models that used triacylglycerols, and then examined as anti-isomerizing reagents. Finally, the effects of the heating and frying processes on the induction of *trans* fatty acids were investigated by using commercially available edible oils.

The purpose of this study was to obtain fundamental information about the formation of *trans* fatty acids in edible oils during a domestic or commercial cooking process through the model systems.

Formation of *trans* fatty acids in heated triacylglycerols

In addition to their dietary resources, *trans* fatty acids are endogenously produced in animal tissues from natural *cis* isomers via oxidative stress generation under physiological and pathological conditions.[9] The free radical-catalyzed *trans* isomerization of natural unsaturated lipids has already been investigated, and isomerization induced by radical species using an addition-elimination mechanism has been proposed. The bisallylic hydrogen in unsaturated fatty acids is also abstracted during thermally induced lipid oxidation, and the oxidative cleavage of double bonds forms radical species. Previous studies have reported that the continuous heating of vegetable oils produced a small amount of *trans* fatty acids.[2,5,8] We also investigated the effect of thermally induced oxidative stress on the *cis-trans* isomerization of double bounds in lipids, using highly purified triacylglycerols. Triolein (*cis-9*; 0.5 g) was heated at 160ºC, 180ºC, 200ºC, and 220ºC. The accumulations of *trans* isomers (Fig. 1 A) and residual *cis* isomers (Fig. 1 B) were measured to monitor the isomerization rate and oxidative degradation rate of double bonds in triolein, respectively. The *trans* isomerization of the *cis* double bonds in triolein was dependent on heating temperature and heating period. In

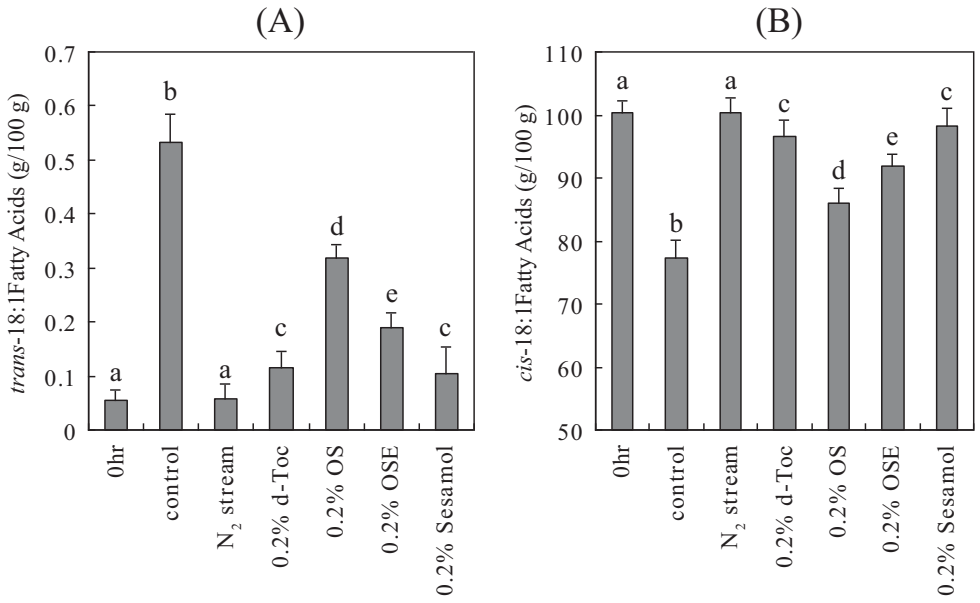

Fig. 2. Amounts of *trans* 18:1 isomers (A) and residual *cis* 18:1 isomers (B) in triolein after heating at 180°C for four hours
The values represent the means ± SD of three experiments. The values at different antioxidants not sharing a common letter differ significantly ($P<0.05$).

contrast, the rate of oxidative degradation at each examined temperature was considerably larger than that of *trans* isomerization, as shown in Fig. 1.

The addition of such antioxidants as δ-tocopherol (δ-Toc, 0.2%), rosemary extract (OS, 0.2%), a mixture of rosemary extract and mixed tocopherol (OSE, 0.2%), and sesamol (0.2%) suppressed the *cis*-to-*trans* isomerization of double bonds in triolein at different levels (Fig. 2 A). These antioxidants also reduced the degradation rate of *cis* double bonds in triolein (Fig. 2 B). The inhibitory effect of the antioxidants on both heat-induced *trans* isomerization and *cis*-degradation of the double bonds in triolein increased in order of sesamol (0.2%), δ-Toc (0.2%), OSE (0.2%), and OS (0.2%). However, the inhibitory effect of these antioxidants on the *trans* isomerization process was incomplete, compared with that produced by substituting ambient oxygen with nitrogen (from the N_2 steam), as shown in Fig. 2 A.

When trilinolein (*cis-9, cis-12*, 18:1; 0.5 g) was heated at 180°C for four hours, only a very little amount of *trans* 18:2 isomers (mainly *trans-9, cis-12*, 18:2 and *cis-9, trans-12*, 18:2) was accumulated (data not shown). The effects of antioxidants on suppressing the heat-induced *trans*-isomerization of the double bonds in trilinolein were detectable but not marked, as small amounts of *trans* isomers accumulated in trilinolein, even in the absence of antioxidants. In contrast, the thermal oxidative degradation rates of the *cis* double bonds in trilinolein were larger than those of the *cis* double bonds in triolein. The inhibitory effect of the antioxidants on thermal *cis* degradation of the double bonds in trilinolein increased in order of 0.2% sesamol, δ-Toc, OSE, and OS. More than 95% of the *cis*-double bonds were intact in trilinolein heated in the presence of 0.2% sesamol for four hours at 180°C.

During thermal oxidation, unsaturated fatty acids may undergo hydrogen abstraction and form corresponding radicals. Being thermodynamically unstable, the radicals are immediately converted into peroxyl radicals in the subsequent oxidative process. Antioxidants react with peroxyl radicals and form a stable compound, thereby interrupting further lipid oxidation. In the presence of nitrogen, oxygen does not interact with the radicals generated from the *cis* double bond, thereby interrupting the formation of peroxyl radicals and terminating the free-radical chain reaction. Therefore, the addition of antioxidants and substitution of nitrogen suppresses the successive supply of lipid radicals. Thus, a supplement of lipid radicals is indispensible for the heat-induced *trans* isomerization of triolein. Our results suggest that triolein radicals are intermediate species formed in heat-induced *trans* isomerization.

One previous study suggested that the isomerization of oxidative stress-induced lipids was potentially important in aging and disease, because *trans* fatty acid chains could affect both membrane properties and function.[9] The isomerization of double bonds in various unsaturated fatty acids will require further physical analysis as

pertaining to not only food processing but also research related to human health.

Formation of *trans* fatty acids in heated edible oils

Refined edible oils derived from various plants are commercially available and occasionally used in the frying process. These edible oils contain a small amount of *trans* fatty acids (0-2%) that were produced during the purification process of crude oil. To estimate the formation of *trans* fatty acids in edible oils during cooking, six commercially available edible oils were heated in the simple model system without food and the profile of *trans* fatty acid formation was investigated. As listed in Table 1, the total amount of *trans* fatty acids (sum of *trans* fatty acids above the limit of quantitation (QL: 0.047 g/100 g oil)) in these fresh oils was in the range of 0.26 g to 1.54 g/100 g. Prior to heating, the cooking oil, canola oil, and corn oil mainly contained *trans* 18:2 and *trans* 18:3, while *trans* 18:1 and *trans* 18:2 were predominantly detected in fresh safflower oils and fresh sesame oil (Table 1). These *trans* isomers in the fresh oils are typically produced in the deodorization process of crude oil. The edible oil (1.0 g) was heated at 180°C for four hours in a small glass tube. Figure 3 shows the GC chromatograms of fresh canola oils and related heat treatment (at 180°C for four hours). After four hours of heat treatment at 180°C in the model system, small increases were observed in *trans* 18:1 in safflower oil and rice bran oil (from 0.05 g/100 g to 0.14 g/100 g, and from 0.09/100g to 0.11 g/100 g, respectively), and in *trans* 18:1 (from 0.14 g/100 g to 0.25 g/100 g) and *trans* 18:2 (from 0.32 g/100 g to 0.59 g/100 g) in sesame oil. In contrast, *trans* 18:3 in cooking oil, corn oil, and rice oil decreased slightly depending on the period of heating.

Substantial increases were previously observed in *trans* 18:2 and *trans* 18:3 in safflower oil heated at 250°C – 350°C.[2,6] Those past studies also suggested that no significant production of *trans* isomers of these polyunsaturated fatty acid was detected when corresponding oils were heated at less than 200°C. In this study, *trans* 18:2 and *trans* 18:3 in commercially available edible oils did not form substantially by heating at 180°C. Thus, our results support those of the previous studies.[2,5,8]

One previous study reported a linear relation between the amounts of *trans* 18:1 in several vegetable oils and the heated periods.[2] This feature could be found in *trans* 18:1 in the heated oils investigated by this study. As listed in Table 1, statistically significant differences ($P<0.05$) were observed in the amounts of *trans* 18:1 in heated rice bran oil, safflower oil and sesame oil, as compared with the fresh ones. However, these increases in *trans* 18:1 produced by heating were too small to affect the dietary intake of *trans* fatty acids from these oils.

The fatty acid composition of each vegetable oil depends on the plant from which it is derived. Moreover, the antioxidants coexisting in these vegetable oils were diverse in terms of kind and concentration. These factors—the fatty acid composition of each oil and the coexisting antioxidants—contribute to variations in the profiles of accumulated heat-induced *trans* fatty acids.

Table 1. Formation of *trans* fatty acids in the edible oils by heating (180°C)

	heated period (hr)	C18:1t (g/100g)	C18:2t (g/100g)	C18:3t (g/100g)	Total (g/100g)	Total >LQ (g/100g)
Cooking oil	0	(0.02)	0.37	1.06	1.45	1.43
	4	0.08	0.37	0.97	1.42	1.42
Canola oil	0	(0.04)	0.13	0.86	1.03	0.99
	4	0.06	0.14	0.89	1.09	1.09
Corn oil	0	(0.04)	1.21	0.33	1.58	1.54
	4	0.06	1.21	0.31	1.58	1.58
Rice bran oil	0	0.09	0.70	0.33	1.12	1.12
	4	0.11	0.69	0.31	1.11	1.11
Safflower oil	0	0.05	0.21	(0.04)	0.3	0.26
	4	0.14	0.19	(0.04)	0.37	0.33
Sesami oil	0	0.14	0.32	(0.01)	0.47	0.46
	4	0.25	0.59	(0.02)	0.86	0.84

( ) denotes peak intensity less than the quantitation limit (QL).

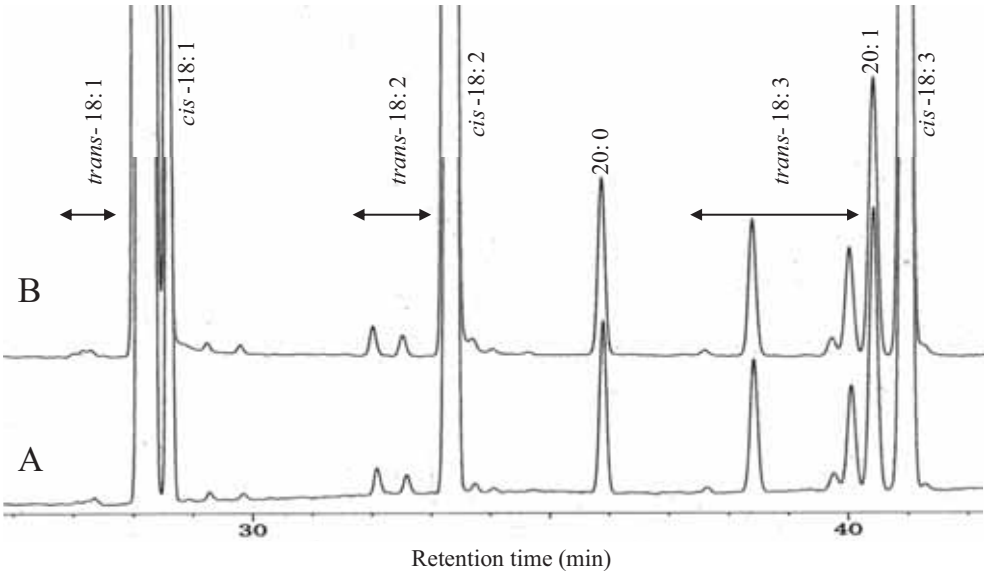

Fig. 3. Gas chromatogram of fatty acid methyl esters of fresh canola oil (A) and canola oil heated at 180°C for four hours (B)

Formation of *trans* fatty acids in the frying process

The formation of *trans* fatty acids in oil during the frying process has also been investigated.[1,2] Among these studies, the degree of *trans* fatty acid formation during frying depended on the frying conditions and frying materials. When such frying materials as pre-fried frozen potatoes containing *trans* fatty acids were introduced to the frying process, *trans* fatty acids released from the materials into the frying oil during frying should be considered. The formation of *trans* fatty acids in frying oil caused by the frying process itself should be distinguished from the *trans* fatty acids initially existing in the frying materials. Foods containing very little *trans* fatty acids are recommended as frying test materials. In addition, more detailed monitoring of *trans* fatty acids accumulated in both the frying materials and frying oil is needed to investigate the formation of *trans* fatty acids in edible refined oils under domestic or commercial cooking conditions.

In this study, the formation of *trans* fatty acids in edible oils during the frying process was investigated by using fresh raw potatoes and commercially available corn oil as the frying materials and frying oil, respectively. Potatoes harvested in Hokkaido, Japan, were purchased from a local market and stored at room temperature until use. Raw potatoes (100 g) were sliced into pieces (1.0 × 1.0 cm in thickness and 8 to10 cm in length), and then fried in edible corn oil. (Note the amount of each *trans* fatty acid differed slightly as the corn oil used in this experiment and that used in the heating experiment came from different lots of production.) After every tenth frying operation at 180°C, the *trans* fatty acids in the frying oils were analyzed. The amounts of *trans* 18:1 in fresh corn oil were less than the LQ. Even after the 30th frying operation at 180°C, their amounts were still less than the LQ. Figure 4 shows the amounts of *trans* 18:1, *trans* 18:2, and *trans*18:3 in the frying corn oil at every tenth frying operation. Throughout the frying operations, the accumulation of these *trans* fatty acids was not distinguished statistically from those in the fresh oil (Fig. 4).

The lipid content in the raw potatoes was 0.11% (w/w), and there were undetectable *trans* isomers of unsaturated fatty acids in the lipid (data not shown). Conversely, the lipid content of potatoes fried at 180°C was 8.8 ± 0.1%. No difference was found in lipid content depending on the number of frying operations. The fatty acid composition of the fried potatoes was consistent with that of the frying oil, rather than with that of the original raw potatoes, thus suggesting that most lipids composed of fried potatoes were transferred from the frying oil.

Based on the amounts of *trans* fatty acids in the frying oils and the lipid content in the fried potatoes, the intake of *trans* fatty acids was calculated. When 100 g of potatoes fried at 180°C in this study were served, the range of *trans* fatty acids intake was estimated at 0.129 ± 0.003 g. The repeated frying process would not have a large effect on *trans* fatty acid intake from the fried potatoes. As described in the heating model of edible oils, the formation of *trans* fatty acids in canola oil, cooking oil, rice bran oil, safflower oil, and sesame oil during

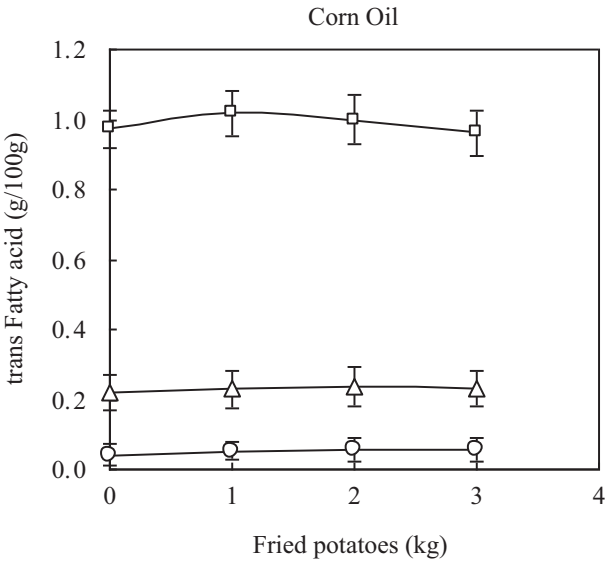

Fig. 4. Amounts of *trans* 18:1, *trans* 18:2 and *trans* 18:3 in corn oil after every 10th frying operation (with 100 g of potatoes fried per frying operation)

Although the amounts of *trans* 18:1 in corn oils were less than the LQ throughout the frying operations, this figure shows related changes. The values represent the means ± SD of three experiments.

—○— : trans-18:1, —□— : trans-18:2, —△— : trans-18:3.

heating was not remarkable, and matched the same level as that of corn oil. Based on these results, introducing other oils as frying oils would not significantly change the concentration of *trans* fatty acids in these edible oils. We are now studying the exact formation of *trans* fatty acids during frying by using other edible oils.

In recent studies conducted with extremely precise gas chromatography analysis conditions, a higher amount of elaidic acid was observed in frying oil than in heated oil, thus suggesting the release of *trans* 18:1 from pre-fried frozen foods into the oil during frying.[1,2] This study introduced raw potatoes containing undetectable *trans* fatty acids as frying materials, and revealed that no *trans* 18:1 isomers including elaidic acid accumulated in the corn oil during frying. The results suggest that the frying process itself hardly contributes to the accumulation of particular elaidic acids in frying oils.

It is therefore possible to conclude from this study that heating and frying processes using unhydrogenated edible oils have little impact on the dietary intake of *trans* fatty acids.

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