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cis/*trans*-Isomerisation of triolein, trilinolein and trilinolenin induced by heat treatment

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Abstract

To estimate the *trans*-fatty acid production of edible oils during the frying process, 1.0 g of triolein, trilinolein and trilinolenin, as representative oils, were heated at 180 °C for a defined period. The amounts of *trans*-fatty acids in heated triacylglycerols were quantitatively determined by gas chromatography after methylation. It was revealed that heating induced *cis* to *trans*-isomerisation of unsaturated triacylglycerols, and that *trans*-fatty acid amounts increased gradually, depending on the heating period. For example, *trans*-isomer amounts in triolein, trilinolein and trilinolenin (per gram) were 5.8 mg, 3.1 mg and 6.5 mg, respectively, after 8 h incubation at 180 °C. At that time, the contents of polar compounds contained in the heated triolein, trilinolein and trilinolenin were 22%, 27% and 31%, respectively. When triolein was heated under a N_2 stream, neither *trans*-isomerisation nor polar compounds were detected. The addition of α-tocopherol (1.0%) to triolein significantly prevented not only lipid oxidation but also *trans*-isomerisation during heating. A commercially available vegetable oil was also heated under the same conditions as these model oils. Compared with the *trans*-isomerisation in model oils, the degree of *trans*-isomerisation in the edible oil was relatively low. Tocopherols in the oil would prevent not only lipid oxidation but also isomerisation. These results suggest that the geometric isomerisation of unsaturated fatty acids during heating accompanies lipid oxidation.

Keywords: *trans*-Fatty acid; Triacylglycerol; Heat treatment

1. Introduction

The interest in *trans*-fatty acids has increased in the past ten years, because of the relation between *trans*-fatty acid intake and the risk of cardiovascular disease (Dausch, 2002; Stender & Dyerberg, 2004). Although *trans*-fatty acids of unsaturated lipids are not natural lipid isomers, *trans*-fatty acids can be induced from *cis*-unsaturated fatty acids in a number of ways. Several bacteria, especially parasitic bacteria in the rumen of cattle, can convert unsaturated lipids from *cis*- to *trans*-isomers through a *cis*/*trans* isomerase (Heipieper, Meinhardt, & Segua, 2003; Loffeld & Keweloh, 1996; Ramos et al., 1997). Partial hydrogenation of fats or oil induces the *cis* to *trans*-isomerisation of double bonds, in addition to the conversion of double bonds to single bonds and the shifting of double bonds along the carbon chain. Refined edible vegetable oils contain low quantities of *trans*-fatty acids. However, heat treatments, such as the frying process, have produced diverse amounts of *trans*-fatty acid depending on the oils used (Bharati, Rostum, & Loberg, 1994; Liu, Inbaraj, & Chen, 2007; Romero, Cuesta, & Sanchez-Muniz, 2000). The isomerisation mechanism of unsaturated fatty acids by heating should be investigated. Furthermore, *trans*-fatty acids production during cooking should be considered, to evaluate the exact intake of *trans*-fatty acids in the diet. In this study, 1.0 g of highly purified triolein, trilinolein and trilinolenin, as representative oils, were incubated at 180 °C, to simulate the frying process, and *trans*-fatty acid production was determined. In addition to these model oils, commercially available edible oil was examined for *trans*-isomerisation by heat treatment.

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2. Materials and methods

2.1. Materials

Triolein, trilinolein and trilinolenin of chemical pure grade were purchased from Funakoshi Co. Ltd. (Tokyo, Japan). The *cis*-and *trans*-fatty acid methyl ester standards with 1–3 double bonds, including oleic acid methyl ester (C18:1, *cis*-9), elaidic acid methyl ester (C18:1, *trans*-9), *cis*-linoleic acid methyl ester (C18:2, *cis*-9, *cis*-12), linoleic acid isomer methyl esters (C18:2, *cis*-9, *cis*-12; *cis*-9, *trans*-12; *trans*-9, *cis*-12; *trans*-9, *trans*-12), *cis*-linolenic acid (C18:3, *cis*-9, *cis*-12, *cis*-15) and linolenic acid isomer methyl esters (C18:3, *cis*-9, *cis*-12, *cis*-15; *trans*-9, *cis*-12, *cis*-15, *cis*-9, *trans*-12, *cis*-15; *cis*-9, *cis*-12, *trans*-15; *trans*-9, *trans*-12, *cis*-15; *trans*-9, *cis*-12, *trans*-15; *cis*-9, *trans*-12, *trans*-15; *trans*-9, *trans*-12, *trans*-15) were obtained from Supelco (Bellefonte, PA, USA). α-Tocopherol was commercially available from Merck KGaA (Darmstadt, Germany). *n*-Hexane, diethyl ether and other solvents of analytical grade were obtained from Wako Pure Chemical Co. (Osaka, Japan) and Kanto Chemical Co. (Tokyo, Japan).

2.2. Heating procedures

One gram of each triacylglycerol was transferred to a 10 ml glass tube, and incubated in a silicon oil bath (OHB-1000S, Tokyorika Co. Ltd., Tokyo, Japan) with continuous stirring (RCX-1000D, Tokyorika Co. Ltd.). Silicon oil was obtained from Wako Pure Chemicals Co. (Silicon Oil WF-30). The accuracy of temperature control during heating oil was ±0.2 °C.

2.3. Fatty acid determination

Analysis of fatty acids was carried out by gas chromatography. After heating, about 100 mg of each triacylglycerol was transferred to a small vial with 10 mg of tripalmitin as an internal standard. Methylation was accomplished with a BF_3-Methanol Kit (Supelco). The esters were extracted into hexane and dried under nitrogen. The *trans*-fatty acid content of each triacylglycerol was analysed by gas chromatography (Shimadzu GC-17A, Kyoto, Japan) on a capillary column (Chrompack CP-Sil 88, 50 m × 0.25 mm × 0.2 μm) with helium as the carrier gas (1.0 ml min^{-1}). The temperature of the column was set at 175 °C. Methyl esters of fatty acids were identified by comparison with the retention time of an authentic standard sample. The absolute amounts of *trans*-fatty acids produced by heating were calculated relative to the peak intensity of the internal standard.

2.4. Polar compounds determination

Polar compounds are formed in triacylglycerols during heating. Heated oils were separated by column chromatography into nonpolar and polar compounds according to the AOCS official method Ce 2-66. The polar content was determined by Iatroscan (MK-6, Mitsubishi Chemical. Co. Ltd. Tokyo, Japan), according to the previous method (AOAC, 1990). The measurements were repeated eight times for each sample.

2.5. Tocopherols determination

Tocopherol isomers contained in edible oil were determined by the fluorescence-HPLC method described previously (Taylor & Barne, 1981).

2.6. Statistical analyses

All the experiments were performed in more than triplet and the data were means ± SD. The data were subject to analysis of variance and Duncan's multiple range test for comparison of significant difference ($p < 0.05$).

3. Results

3.1. trans-Fatty acid evolution by heat treatment

One gram each of triolein, trilinolein and trilinolenin were incubated at 180 °C for 2, 4 and 8 h. After heat treatment, the production of *trans*-fatty acids isomerised from corresponding *cis*-unsaturated fatty acids was determined by GC. Before heat treatment, no *trans*-isomers were found in triolein and trilinolenin. But *trans*-isomers of trilinolein sometimes appeared, depending on the sample lots used. The *trans*-isomer contents of these three triacylglycerols increased with an increase in the heating period, as shown in Fig. 1. The *trans*-isomerisations of triolein and trilinolenin by heat treatment were larger than that of trilinolein under the examined conditions. For example, the *trans*-isomer amounts per 1.0 g of triolein, trilinolein and trilinolenin after the 8 h incubation at 180 °C were 5.8 mg, 3.1 mg and 6.5 mg, respectively.

During incubation at 180 °C under atmospheric conditions, lipids are subjected to oxidation and deterioration. As a result, polar compounds are produced. The degree of oxidative deterioration of the triacylglycerol was evaluated by the relative content of residual nonpolar compounds to polar ones. As shown in Fig. 2, the content of nonpolar compounds decreased with an increase in the incubation period. For example, the contents of polar compounds contained in the triolein, trilinolein and trilinolenin, heated for 8 h, were 22%, 27% and 31%, respectively. The results suggested that triolein was most resistant to heat deterioration among the three triacylglycerols examined (Fig. 2). Geometric isomerisation of triolein from *cis* configuration to *trans* by heating reached a level as high as that of trilinolenin (Fig. 1), while isomerisation of trilinolein occurred at the lowest level, although trilinolein was as sensitive to heat deterioration as trilinolenin (Figs. 1 and 2). In terms of conjugated double bond systems, trilinolein should form an intermediate which is more rigid

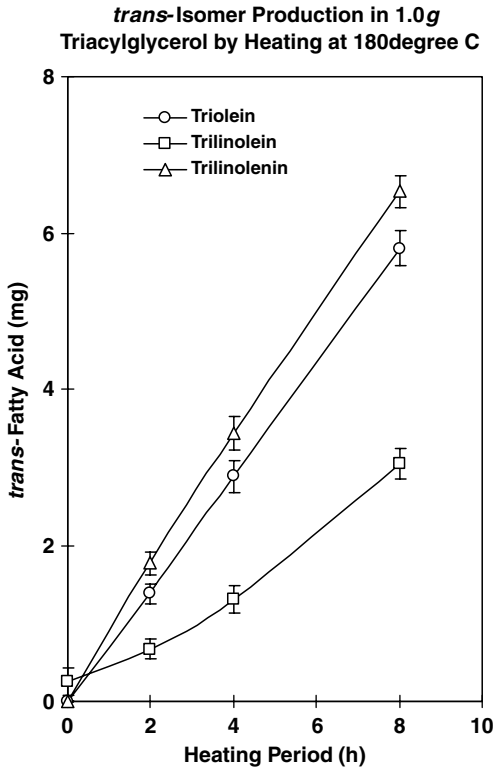

Fig. 1. Time course of the amounts of *trans*-isomers in triolein (○), trilinolein (□) and trilinolenin (△). One gram of each triacylglycerol was incubated in a silicon oil bath at 180 °C.

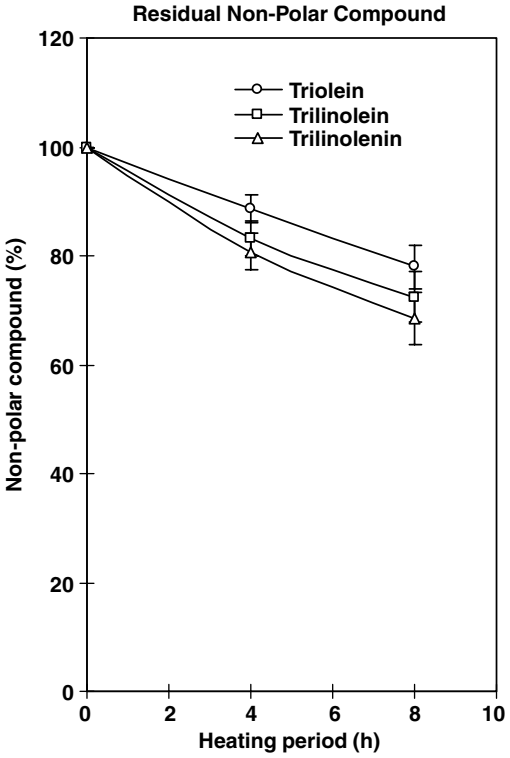

Fig. 2. Time course of residual contents of nonpolar compounds in triolein (○), trilinolein (□) and trilinolenin (△). One gram of each triacylglycerol was incubated in a silicon oil bath at 180 °C.

and thus less prone to geometric isomerisation from *cis* to *trans*.

3.2. Effect of heating temperature

One gram each of triolein, trilinolein and trilinolenin were incubated at 220 °C or 180 °C for 4 h and isomer formation was analysed quantitatively and qualitatively for each triglycerol. At the higher incubation temperature, triacylglycerols were observed to have larger amounts of *trans*-isomerisation of unsaturated bonds (Fig. 3). After 4 h incubation of triolein (1.0 g) at 180 °C and 220 °C, *trans*-isomers constituted 2.9 mg and 7.4 mg, respectively. The same tendency was found in trilinolenin heated at these two temperatures. However, in the *trans*-fatty acid amount of trilinolein when incubated at 180 °C or at 220 °C was smaller than those of triolein and trilinolenin (Fig. 3). The *trans*-isomerisation profiles of these three triacylglycerols with heating at the different temperatures were traced in detail. Based on the GC chromatogram, an isomer with 9-*trans*- and 12-*trans*-isomers was detected in trilinolein incubated at 180 °C (Fig. 4). On the other hand, isomers with 9-*trans*-, 12-*trans*-, and 15-*trans*-structures were characteristically found in trilinolenin after heating at 220 °C (Fig. 4). These results suggested that the heat treatment at high temperature not only induced a large amount of *trans*-isomers but also multiple *trans*-conversions within individual polyunsaturated fatty acids in the side chain of triacylglycerols.

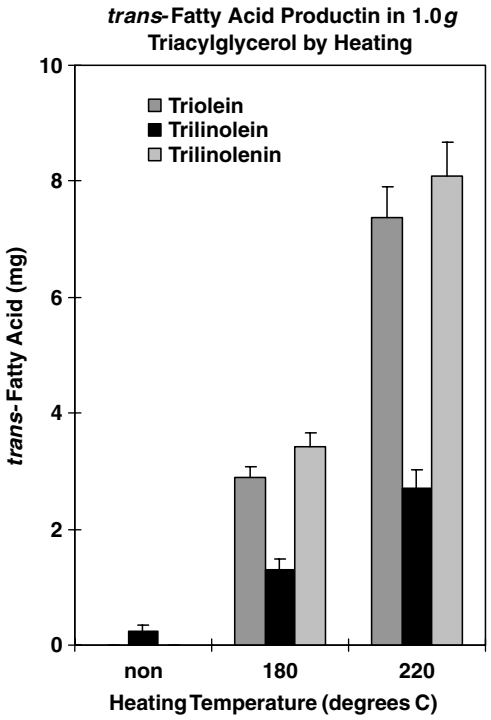

Fig. 3. The amount of accumulated *trans*-isomers of triolein (▩), trilinolein (■) and trilinolenin (▩), when 1.0 g of each triacylglycerol was incubated at 180 °C or 220 °C for 4 h.

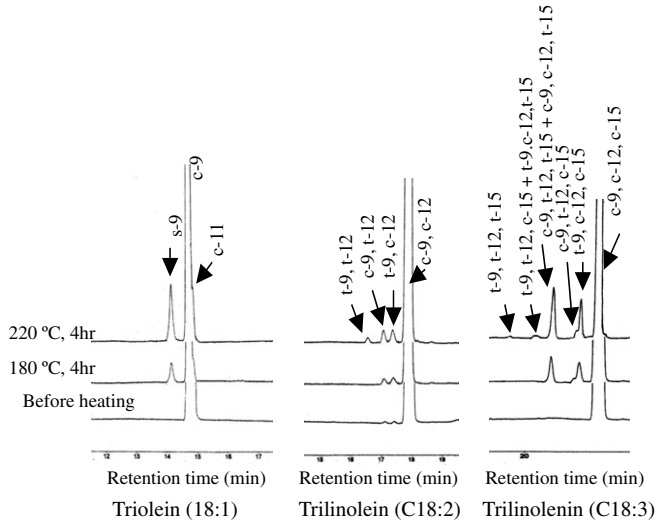

Fig. 4. Gas chromatogram of *trans*-isomers produced by heat treatment at 180 °C and 220 °C for 4 h.

3.3. Factors which induced trans-isomerisation

To elucidate what exactly induced the *trans*-isomerisation of unsaturated fatty acids, triolein (1.0 g) was incubated at 180 °C under several conditions. When the incubation occurred under a N_2 stream, no *trans*-isomerisation occurred, as shown in Fig. 5. The addition of 1.0% α-tocopherol, a lipophilic antioxidant, also considerably prevented the *trans*-formation of triolein heated at 180 °C. Decrease in *trans*-fatty acid formation by the N_2 stream and α-tocopherol suggested that the continuous production of lipid radicals in the chain oxidation process is surely related to the isomerisation of double bond configurations by heat treatment.

Next, we checked the participation of polar compounds produced from lipid deterioration by heat treatment in *trans*-isomerisation of unsaturated bonds. After triolein (1.0 g) was incubated at 180 °C for 8 h, the polar compound content as determined by GC-FID was about 22% (Fig. 2). A small volume of 8 h incubated triolein (0.05 g) was added to fresh triolein (9.95 g), so the amount of *trans*-fatty acid was 0.29 mg before heating. Heat treatment was conducted as usual. As shown in Fig. 5, there was no difference in *trans*-isomers production of triolein with and without polar compounds. This showed that the polar compounds in a triacylglycerol did not affect *trans*-isomerisation during heating.

Together, these findings indicate that isomerisation of unsaturated triacylglycerol by heat treatment could be accompanied by a lipid oxidation reaction, but that polar compounds did not catalyze the *cis*/*trans*-isomerisation of unsaturated bonds.

3.4. trans-Fatty acid evolution of a commercially available edible oil by heat treatment

One gram of commercially available rapeseed oil was incubated at 180 °C for 4 and 8 h. After heat treatment,

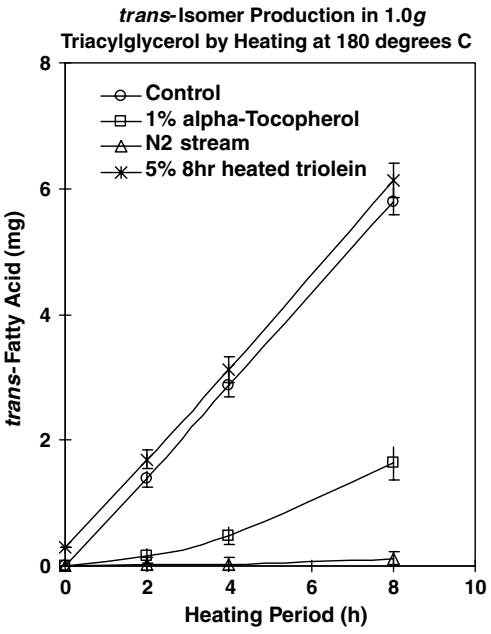

Fig. 5. Time course of the amounts of *trans*-isomers of triolein (○), triolein with 1% α-tocopherol (□), triolein under a N_2 steam (△) and triolein with 5% 8 h-heated triolein (✳). One gram of triolein was incubated in a silicon oil bath at 180 °C.

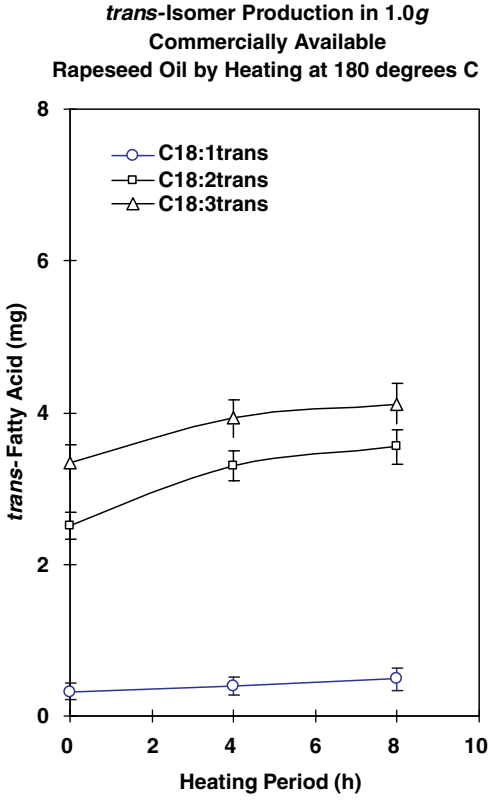

Fig. 6. Time course of the formation of *trans*-isomers in *trans*-C18:1 (○), *trans*-C18:2 (□) and *trans*-C18:3 (△). One gram of commercially available rapeseed oil was incubated in a silicon oil bath at 180 °C.

the production of *trans*-fatty acids from C18:1*cis*, C18:2*cis* C18:3*cis* was determined by GC. Before heat treatment, some *trans*-isomers were found in C18:2 and C18:3. The *trans*-isomer contents of the three unsaturated fatty acids increased with an increase in the heating period, as shown in Fig. 6. However, the *trans*-isomerisations of these unsaturated fatty acids in edible oil by heat treatment were considerably smaller than those of triolein, trilinolein, trilinolenin under the same heating conditions (Fig. 1). The content of tocopherols in the rapeseed oil was determined by the fluorescence-HPLC method described above, and it revealed that the tocopherols content of the oil was 48 mg per 100 g after 8 h heated. Tocopherols would prevent not only rapid oxidation of the edible oil but also *trans*-isomerisation.

4. Discussion

Fried foods are the main sources of *trans*-fatty acids in the human diet. However, most *trans*-fatty acids in these foods have been considered to come from the oil used and not from the process itself. To confirm this, three triacylglycerols, as representative oils, were heated, and the *trans*-fatty acid production was investigated. The most significant finding in this study was that *trans*-isomerisation of unsaturated double bonds by heating coincided with lipid oxidation. The *trans*-isomerisation would have occurred as a side reaction of chain lipid oxidation. The addition of α-tocopherol and a N_2 steam contributed to preventing the continuous production of lipid radicals, and hampered the formation of *trans*-isomers, as described above. This indicates that a supply of lipid radicals from the chain lipid oxidation reaction was necessary for *trans*-isomer production during heating. It also showed that polar compounds themselves did not affect the *trans*-isomerisation of triacylglycerols. In a previous study, *trans*-fatty acids appeared in the tissue of rats fed a diet completely free of *trans*-isomers. That study concluded that lipid radicals led to the formation of *trans*-isomers detected *in vivo* (Zambonin et al., 2006). Furthermore, sulfur-containing compounds induced *cis*/*trans*-isomerisation of unsaturated phospholipids *via* a radical mechanism both in solution and in the phospholipids vesicle (Chatgilialoglu, Ferreri, Ballestri, Mulazzani, & Landi, 2000; Ferreri, Costantino, Landi, Mulazzani, & Chatgilialoglu, 1999). In the latter case, thiyl radicals were concluded to be the species likely to be responsible for the induction of the isomerisation of double bonds in the side chain. These previous studies supported our hypothesis that lipid radicals supplied from lipid oxidation during heating would be the most effective species for isomerising unsaturated bonds. This mechanism of *trans*-formation by heat treatment is now under investigation in our laboratory.

Next, the relation between lipid deterioration by oxidation and *trans*-isomerisation of its double bonds should be considered. In the case of 1.0 g triolein, 8 h heat treatment resulted in 22% (220 mg) polar compounds production (Fig. 2) and 5.8 mg *trans*-fatty acid production (Fig. 1). The ratio of *trans*-isomerisation to lipid oxidation was 1–38. This ratio was as low as 1–88 in the case of heated trilinolein. This difference in the isomerisation/oxidation ratio between triolein and trilinolein suggested that the lipid radicals coming from triolein proceeded to *trans*-isomerisation more easily than those of trilinolein.

We also investigated the *trans*-isomerisation of commercially available edible oil (rapeseed oil) by heat treatment and found the increase in *trans*-fatty acids content was small compared with those of triolein, trilinolein and trilinolenin in model oils. This result was in good accordance with that of a previous study (8). Most edible oils contain tocopherols, which are antioxidants. Prevention of rapid oxidation of the edible oils by intinsic natural antioxidants would result in a small amount of *trans*-isomerisation in each unsaturated fatty acid.

The amounts of newly-formed *trans*-isomers derived from the heating these triacylglycerols were not larger (less than 10.0 mg g^{-1} (<1.0%) even after heating for 8 h) compared with those of partially hydrogenated oils. However, considering the production mechanism of *trans*-fatty acids in the heating process, more attention should be paid to the frying process of foods, to avoid the risk of diseases to which *trans*-fatty acids contribute. Currently, we are investigating the effects of several fried foods on *trans*-fatty acid formation in edible vegetable oils during the frying process.

References

AOAC (1990). Official methods of analysis (15th ed.), 982.27 Arlington, V.A.

Bharati, S., Rostum, G. A., & Loberg, R. (1994). Calibration and standardization of Iatroscan (TLC-FID) using standards derived from crude oils. *Organic Geochemistry, 22*, 835–862.

Chatgilialoglu, C., Ferreri, C., Ballestri, M., Mulazzani, Q. G., & Landi, L. (2000). *cis*/*trans*-Isomerisation of monounsaturated fatty acid residues in phospholipids by thiyl radicals. *Journal of American Chemical Society, 122*, 4593–4601.

Dausch, J. G. (2002). *trans*-Fatty acids: A regulatory update. *Journal of the American Dietetic Association, 102*, 18–20.

Ferreri, C., Costantino, C., Landi, L., Mulazzani, Q. G., & Chatgilialoglu, C. (1999). The thiyl radical-mediated isomerizaion of cis monounsaturated fatty acid residues in phospholipids: A novel path of membrane damage? *Chemical Communications*, 407–408.

Heipieper, H. J., Meinhardt, F., & Segua, A. (2003). The *cis-trans* isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: Biochemistry molecular biology and physiological function of a unique adaptive mechanism. *FEMS Microbiology Letters, 229*, 1–7.

Liu, W. H., Inbaraj, B. S., & Chen, B. H. (2007). Analysis and formation of *trans*-fatty acids in hydrogenated soybean oil during heating. *Food Chemistry, 104*, 1740–1749.

Loffeld, B., & Keweloh, H. (1996). *cis*/*trans*-Isomerisation of unsaturated fatty acids as possible control mechanism of membrane fluidity in Pseudomonas putida P8. *Lipid, 31*, 811–815.

Ramos, J. L., Duque, E., Rodriguez-Herva, J. J., Godoy, P., Haidour, A., Reyes, F., et al. (1997). Mechanism for solvent tolerance in bacteria. *Journal of Biological Chemisry, 272*, 3887–3890.

Romero, A., Cuesta, C., & Sanchez-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.

Stender, S., & Dyerberg, J. (2004). Influence of *trans*-fatty acids on health. *Annals of Nutrition and Metabolism, 48*, 61–66.

Taylor, P., & Barne, P. (1981). Analysis for vitamin E inedible oils by high performance liquid chromatography. *Chemistry & Industry, 17*, 722–726.

Zambonin, L., Ferreri, C., Cabrini, L., Prata, C., Chatgilialoglu, C., & Landi, L. (2006). Occurrence of *trans*-fatty acids in rats fed a *trans*-free diet: A free radical-mediated formation? *Free Radical Biology & Medicine, 40*, 1549–1556.