Effect of Triacylglycerol Structures on the Thermal Oxidative Stability of Edible Oil

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ABSTRACT: Different molecular species of TAG were assessed to determine the influence of TAG structure on the thermal oxidative stability of edible oil. TAG containing palmitic acid (16:0, P) as saturated FA (SFA) and oleic acid (18:1, O), linoleic acid (18:2, L), or linolenic acid (18:3, Ln) as unsaturated FA (UFA) were chemically synthesized and then heated at 180 or 150°C. Thermal oxidative stability of TAG was determined by evaluating the resultant UFA, polar compound, FFA, carbonyl compound, polymerized compound, and tocopherol contents. When TAG containing 16:0 and 18:2 in the ratio of 2:1 (mol/mol) were heated at 180°C, a 2:1 (mol/mol) mixture of saturated TAG (PPP) and unsaturated TAG (LLL) was found to be more susceptible to thermal oxidation than PPP/PLL (1:1) and PPL. Similarly, a 2:1 mixture of PPP and OOO or LnLnLn was more unstable toward thermal oxidation than PPO or PPLn, respectively. Thermal oxidative stability of TAG containing SFA and UFA (2:1) was negatively correlated with the moles of UFA in a single TAG molecule. This tendency was also observed at 150°C. From these results, it is suggested that the TAG structure could be one of the factors determining the thermal oxidative stability of edible oil.

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KEY WORDS: Edible oil, frying, linoleic acid, linolenic acid, oleic acid, palmitic acid, thermal oxidation, triacylglycerol, unsaturated fatty acid.

Frying is an excellent and convenient cooking process, and is used worldwide owing to the unique flavor and palatability of fried foods. Consumption of frying oil also improves the absorption of fat-soluble vitamins and provides EFA. However, frying at about 180°C is very deleterious to edible oil, leading to thermal deterioration and nutritional problems. Thus, several methods to prevent thermal deterioration of edible oils have been indicated. One is to improve frying conditions. Fujisaki *et al.* (1,2) found that thermal oxidation was retarded by reducing the oxygen concentration in air contacting the oil to 4%. Negishi *et al.* (3) reported that decreasing the oil surface area in a fryer was effective in retarding the thermal oxidation of vegetable oil during frying. The other method is to modify properties of the oil by reducing the content of PUFA, such as linolenic acid (18:3, Ln), or enriching the content of antioxidants, such as tocopherols. Normand *et al.* (4) reported that the thermal oxidative stability of canola oil was improved by modifying the FA composition to be high in oleic acid (18:1, O) and low in 18:3. Wagner *et al.* (5) found that supplementation with tocopherol and tocotrienol enhanced the thermal oxidative stability of coconut oil.

On the other hand, a recent study found that TAG structure was related to oxidative stability, formation of oxidation products and odors, and general chemical and physical characteristics. Endo *et al.* (6) reported that the autoxidation rate of synthetic TAG containing eicosapentaenoic acid differed according to the TAG molecular species present. Likewise, the autoxidation rate of synthetic TAG containing linoleic acid (18:2, L) and 18:3 depended on the TAG species. The oxidation rates of LnLnL and LLLn were faster than LnLLn and LLnLn, respectively (7). Thus, TAG species may affect the thermal oxidative stability of edible oils.

In this study, different species of TAG containing palmitic acid (16:0, P) as a representative saturated FA (SFA) and 18:1, 18:2, or 18:3 as unsaturated FA (UFA) were chemically synthesized and then heated at 180 or 150°C to assess the effect of TAG structure on the thermal oxidative stability of edible oils.

MATERIALS AND METHODS

Materials. The FA 16:0, 18:1, 18:2, and 18:3; glycerol; 1,2-dipalmitoylglycerol; and 1-monopalmitoylglycerol were purchased from Sigma Chemical Co. (St. Louis, MO). α-, β-, γ-, and δ-Tocopherols were provided by Eisai Co. (Tokyo, Japan). All other chemicals were of reagent grade.

Chemical syntheses of TAG. The following TAG were chemically synthesized, based on the method of Endo *et al.* (6). Tripalmitoylglycerol (PPP) was synthesized by esterification of 16:0 with glycerol in the presence of 4-dimethylaminopyridine and *N*, *N′*-dicyclohexylcarbodiimide as catalysts. Dilinoleoylpalmitoylglycerol (PLL) and dipalmitoyl-linoleoylglycerol (PPL) were prepared from 1-palmitoylglycerol and 1,2-dipalmitoylglycerol, respectively. Chemically synthesized TAG were purified by elution through a Florisil column (prepared in our laboratory), deactivated with 7% water, with diethyl ether/*n*-hexane (7:93, vol/vol) to remove polar compounds such as FFA, carbonyl compounds, and polymerized compounds. The purity of all TAG was higher than 95%

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by HPLC analysis. Other TAG (LLL, LnLnLn, OOO, PPO, POO, PPLn, and PLnLn) were synthesized similarly.

All TAG samples had negligible contents of FFA (<0.1 mg/g), carbonyl compounds (<5 meq/kg), and polar and polymerized compounds (<0.1%).

Heating test. TAG (1 g = 11 mmol) were supplemented with a tocopherol mixture (α, 200 mg/kg; β, 100 mg/kg; γ, 100 mg/kg; δ,100 mg/kg) in a 10-mL test tube and heated at 180 or 150°C. After heating, TAG samples were stored at –30°C until analysis.

Analyses. The contents of UFA, polar compounds, FFA, carbonyl compounds, polymerized compounds, and tocopherols were measured, and DSC analysis was carried out.

Being UFA, 18:1, 18:2, and 18:3 were methylated and then quantified by GC analysis using a CP-Sil 88 column (i.d. 0.25 mm × 50 m; GL Science, Tokyo, Japan). Polar compound contents were determined by the TLC-FID method using Iatroscan TH-10 (Iatron Laboratories, Inc., Tokyo, Japan) with Chromatorod S-III as solid phase and *n*-hexane/diethyl ether/formic acid (80:20:1, by vol) as mobile phase (8). FFA content was measured spectroscopically according to the method of Chakrabarty *et al.* (9). Carbonyl compound contents were determined by the 2,4-dinitrophenylhydrazine method according to Kumazawa and Oyama (10). Polymerized compound contents were measured by high-performance size-exclusion chromatography (HPSEC) after isolation of polar compounds in heated TAG samples (11). Polar compounds were collected from heated TAG samples by passage through a silicic acid column with chloroform/methanol (1:1, vol/vol). The polar compounds were subjected to HPSEC with a dual-jointed Shodex KF-8025 column (5 mm i.d. × 300 mm; Showa Denko Co., Tokyo, Japan). The mobile phase was THF and the flow rate was 1.0 mL/min. Polymerized compounds were detected with a Shodex RI-71 refractive index detector (Showa Denko Co.) at 35°C. Tocopherol contents were determined after saponification according to the standard method of the Japan Oil Chemist's Society (12). For DSC analysis, the onset temperature was measured using a DSC2920 (TA Instruments, New Castle, DE). TAG samples were put in standard open aluminum pans, held at 60°C for 5 min, and then heated to 220°C at 5°C/min (13).

Statistics. All data were described as the average (AVG) and SD of triplicate measurements. Statistical analysis was carried out by Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Thermal oxidation of TAG containing 16:0 and 18:2 (2:1). Four kinds of TAG samples—PPL, PPP/PPL/PLL (1:1:1), PPP/PLL (1:1) and PPP/LLL (2:1)—as TAG containing 16:0 and 18:2 (2:1) were heated at 180°C for 4 to 12 h. Figure 1 shows the content of 18:2 after heating of four TAG samples. The 18:2 content of the four unoxidized TAG samples was 33%, but it fell with heating time. The content of 18:2 in PPP/LLL (2:1) was decreased remarkably in comparison with other TAG samples during heating. The residual amount of 18:2 in PPP/LLL (2:1) was 12% after heating for 12 h. On the other hand, the 18:2 content in PPL declined to 18%, which was significantly higher than the other three TAG samples.

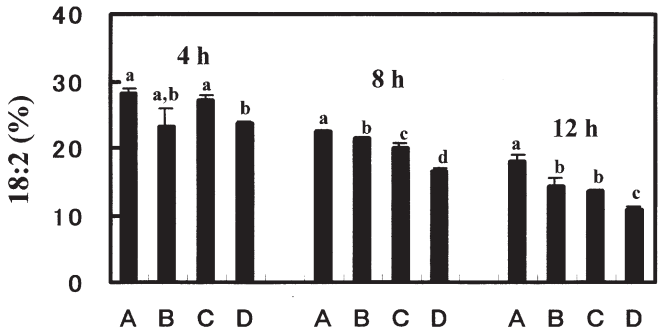

FIG. 1. Linoleic acid contents in TAG containing palmitic acid (16:0, P) and linoleic acid (18:2, L) in a 2:1 ratio after heating at 180°C. Data are presented as average ± SD ($n = 3$).Values with different letters were significantly different ($P < 0.05$). (A) PPL; (B) PPP/PPL/PLL (1:1:1); (C) PPP/PLL (1:1); (D) PPP/LLL (2:1).

Unoxidized TAG samples contained no polar compounds, but considerable amounts of polar compounds formed with heating time, as shown in Figure 2. The content of polar compounds after 12 h of heating in PPL was 40%, which was lower than in the other TAG samples. Polar compounds included DAG and MAG, FFA, aldehydes, alcohols, polymerized compounds, and so on. Therefore, FFA, carbonyl compounds, and polymerized compounds were quantified in TAG samples after heating.

Figure 3 shows the contents of FFA, which were produced by hydrolysis during heating of TAG samples. FFA in all TAG samples was increased with heating time. PPP/LLL (2:1) produced more FFA (60 mg/g) than any other TAG samples (49–53 mg/g) after 12 h of heating.

Carbonyl compounds, mainly produced by the secondary oxidation of hydroperoxides, were remarkably increased with heating time (Fig. 4). PPP/LLL (2:1) showed a significantly higher carbonyl compound content (87 and 141 meq/kg at 4 and 12 h, respectively) than other TAG samples after heating.

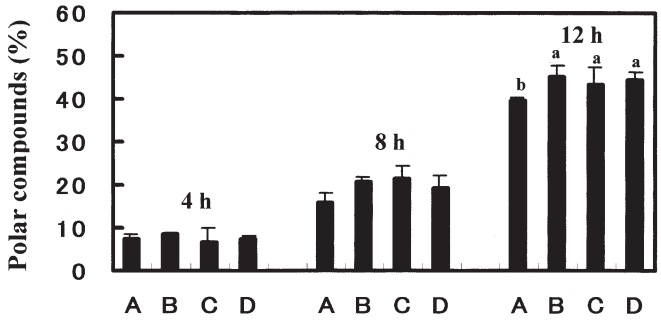

FIG. 2. Polar compound contents in TAG containing 16:0 and 18:2 in a 2:1 ratio after heating at 180°C. Data are presented as average ± SD ($n = 3$). Values with different letters were significantly different ($P < 0.05$). For A–D see Figure 1.

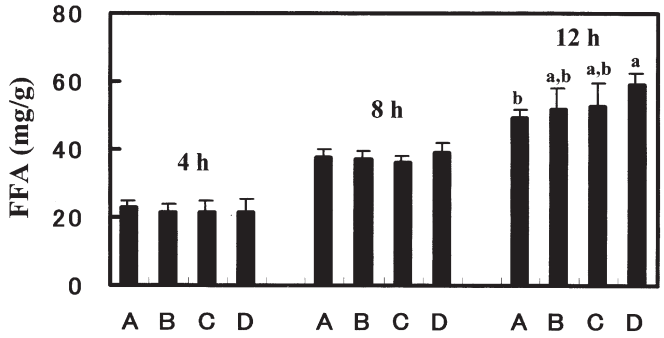

FIG. 3. FFA content in TAG containing 16:0 and 18:2 in a 2:1 ratio after heating at 180°C. Data are presented as average ± SD ($n = 3$). Values with different letters were significantly different ($P < 0.05$). For A–D see Figure 1.

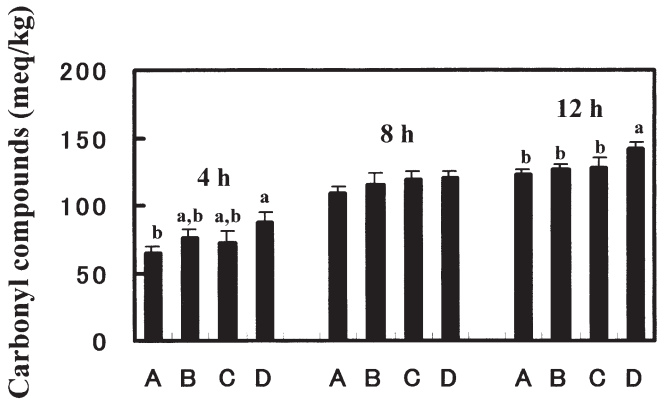

FIG. 4. Carbonyl compound contents in TAG containing 16:0 and 18:2 in a 2:1 ratio after heating at 180°C. Data are presented as average ± SD ($n = 3$). Values with different letters were significantly different ($P < 0.05$). For A–D see Figure 1.

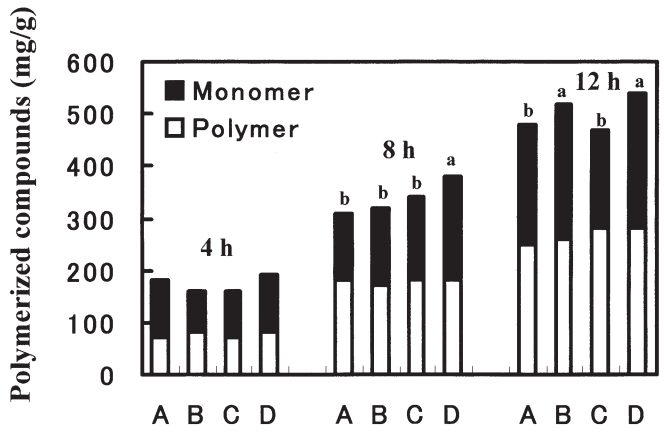

FIG. 5. Polymer and monomer contents in TAG containing 16:0 and 18:2 in a 2:1 ratio after heating at 180°C. Values with different letters were significantly different ($P < 0.05$). For A–D see Figure 1.

Figure 5 shows the content of polymers and monomers after heating of the four TAG samples at 180°C. Considerable amounts of polymers and monomers were formed in all TAG samples after heating. The content of polymers and monomers (370 mg/g) was significantly higher in PPP/LLL (2:1) after heating for 8 h.

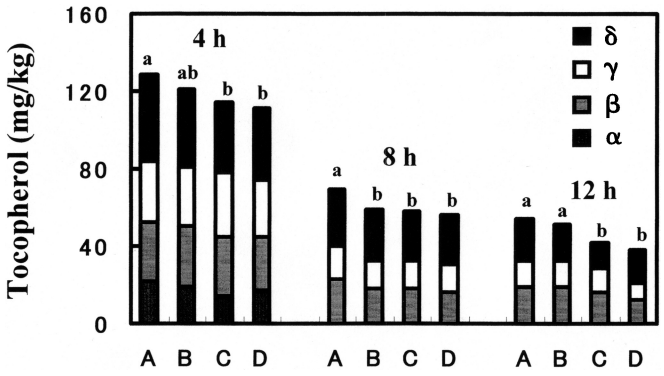

FIG. 6. Tocopherol content in TAG containing 16:0 and 18:2 in a 2:1 ratio after heating at 180°C. Values with different letters were significantly different ($P < 0.05$). For A–D see Figure 1.

Figure 6 shows the tocopherol contents of the four TAG samples after heating at 180°C. The contents of all tocopherol isomers decreased in all TAG samples with heating time. α-Tocopherol completely decomposed in all TAG samples after heating for 8 h. The total tocopherol content was 70 mg/kg in PPL after 12 h of heating, which was significantly higher (56–58 mg/kg) than in other TAG samples. However, there was no significant difference in the tocopherol compositions among the four heated samples.

PPL, PPP/PLL (1:1), and PPP/LLL as TAG containing 16:0 and 18:2 in ratios of 2:1 were heated at 150°C to identify the effect of heating temperature on their thermal oxidation. Table 1 shows the contents of 18:2, polar compounds, FFA, carbonyl compounds, and polymerized compounds of three TAG samples after heating for 8 h. The residual amount of 18:2 was higher in PPL, whereas the contents of carbonyl compounds, polar compounds, and polymerized compounds (polymers and monomers) were higher in PPP/LLL (2:1). These observations were similar to those at 180°C. TAG samples containing highly unsaturated TAG molecular species such as LLL were susceptible to thermal oxidation regardless of heating temperature. TAG structure could affect the thermal oxidative deterioration of TAG containing 18:2.

The onset temperatures of TAG samples, i.e., initiation temperatures of thermal oxidation, as measured by DSC analysis, were 178, 171, and 175°C for PPL, PPP/PLL (1:1), and PPP/LLL (2:1), respectively. Therefore, an oil with a high onset temperature could be stable for the thermal oxidation. PPL had a higher onset temperature than the other three TAG samples containing 16:0 and 18:2 (2:1). This result is similar to that of the chemical characteristics above, and suggests that PPL is more stable than other TAG samples.

Thermal oxidation of TAG containing 16:0 and 18:1 (2:1). Three kinds of TAG samples—PPO, PPP/POO (1:1), and PPP/OOO (2:1)—as TAG containing 16:0 and 18:1 (2:1), also were heated at 180°C. Table 2 shows the contents of 18:1, polar compounds, FFA, and carbonyl compounds in the three TAG samples after 8 h of heating.

The decrease in 18:1 content was observed in all TAG samples after heating. The residual amount of 18:1 differed

TABLE 1
Chemical Characteristics of TAG Containing Palmitic Acid (P) and Linoleic Acid (L) (2:1) After Heating[a] at 150°C for 8 h

	PPL		PPP/PLL (1:1)		PPP/LLL (2:1)	
	AVG	SD	AVG	SD	AVG	SD
Linolic acid (%)	29.6[a]	0.4	27.5[a,b]	0.4	26.9[b]	0.8
Polar compounds	14.1[b]	0.7	16.8[b]	1.7	21.8[a]	1.3
FFA (mg/g)	30.8	5.3	31.8	4.6	32.8	3.1
Carbonyl compounds (meq/kg)	83.5[b]	7.0	118.0[a,b]	6.2	125.4[a]	6.3
HPSEC analysis						
Polymers (mg/g)	101.7[b]	12.7	99.5[b]	10.4	121.7[a]	3.6
Monomers (mg/g)	87.1[b]	5.4	137.4[a]	35.9	145.3[a]	6.8

[a]Data are represented as an average (AVG) and SD of triplicate measurements. Values with different superscript letters were significantly different at $P < 0.05$. HPSEC, high-performance size-exclusion chromatography.

TABLE 2
Chemical Characteristics and Tocopherol Content of TAG Containing Palmitic Acid (P) and Oleic Acid (O) (2:1) After Heating[a] at 180°C for 8 h

	PPO		PPP/POO (1:1)		PPP/OOO (2:1)	
	AVG	SD	AVG	SD	AVG	SD
Oleic acid (%)	23.6[a]	1.4	19.1[b]	0.8	18.6[b]	2.0
Polar compounds (%)	15.5	1.2	17.3	0.4	17.7	0.5
FFA (mg/g)	14.9	1.9	18.4	2.3	19.0	3.1
Carbonyl compounds (meq/kg)	102.8[b]	5.4	111.7[a,b]	11.8	114.2[a]	4.5
Total tocopherols (mg/kg)	54.7[a]	5.7	46.3[a,b]	6.7	41.7[b]	5.6
α-Tocopherol (mg/kg)	0		0		0	
β-Tocopherol (mg/kg)	22.3[a]	1.1	11.3[b]	1.5	14.1[b]	0.7
γ-Tocopherol (mg/kg)	13.2	3.5	14.6	1.8	9.0	2.1
δ-Tocopherol (mg/kg)	19.2	0.9	20.3	3.7	18.6	3.6

[a]For footnote see Table 1.

between the three TAG samples. PPO showed a higher amount of 18:1 than PPP/POO (1:1) and PPP/OOO (2:1) after 8 h of heating.

There was no significant difference in polar compound contents between the three TAG samples, although polar compounds were formed after heating.

FFA were produced in all TAG samples after heating, but the content of FFA produced during heating was not significantly different between the three TAG samples.

Considerable amounts of carbonyl compounds were produced in all TAG samples after heating for 8 h. The increase in carbonyl compounds in particular was higher in PPP/OOO (2:1).

All tocopherol isomers were decreased after heating. No α-tocopherol at all was detected for the three TAG samples heated for 8 h. The contents of total tocopherols and β-tocopherol were higher in PPO than in PPP/POO (1:1) and PPP/OOO (2:1) after 8 h of heating.

These results were similar to those observed for TAG containing 18:2. The thermal oxidative deterioration of TAG containing 18:1 depended on the TAG molecular species that were present. Among the three TAG containing 16:0 and 18:1 in a 2:1 ratio, PPP/OOO was most oxidized during heating at 180°C.

Thermal oxidation of TAG containing 16:0 and 18:3 (2:1). Two kinds of TAG samples—PPLn and PPP/LnLnLn (2:1)—as TAG containing 16:0 and 18:3 (2:1) were heated at 180°C. Table 3 shows the contents of 18:3, FFA, polar compounds, carbonyl compounds, and tocopherols after 8 h of heating of the two TAG samples.

Residual amounts of 18:3 were higher in PPLn after 8 h of heating, but the contents of polar compounds were higher in PPP/PLnLn (2:1). Greater amounts of γ- and δ-tocopherols remained in PPLn than in PPP/LnLnLn (2:1) after heating, but α-tocopherol was not detected in either TAG sample.

These results resembled those in TAG samples containing 18:1 or 18:2. The thermal oxidative deterioration of TAG containing 18:3 was also affected by TAG molecular species, and TAG samples containing highly unsaturated TAG such as LnLnLn were very unstable toward thermal oxidation.

Chemically synthesized TAG that contained SFA and UFA in a 2:1 ratio were heated at 180°C to assess their thermal oxidative stability. Mixtures of saturated TAG (PPP) and highly unsaturated TAG (OOO, LLL, and LnLnLn) were found to be more susceptible to thermal oxidation when TAG samples had the same FA. And the thermal oxidative stability of TAG that contained SFA and UFA (2:1) was negatively correlated with the number of moles of UFA in a single TAG molecule. This tendency was independent of the heating temperature.

In general, the thermal oxidative stability of edible oils depends on their FA composition. The relative oxidizability of FA is 8.6 for SFA, 15.9 for 18:1, 26.8 for 18:2, and 41.5 for 18:3, respectively (14). Thus, the variety and level of UFA in edible oil

TABLE 3
Chemical Characteristics and Tocopherol Contents of TAG Containing Palmitic Acid (P) and Linolenic Acid (Ln) (2:1) After Heating[a] at 180°C for 8 h

	PPLn		PPP/LnLnLn (2:1)	
	AVG	SD	AVG	SD
Linolenic acid (%)	24.4[a]	0.6	21.1[b]	0.6
Polar compounds (%)	26.0[b]	0.8	31.4[a]	0.8
FFA (mg/g)	30.9	1.7	33.2	1.8
Carbonyl compounds (meq/kg)	135.1	7.7	143.5	8.7
Total tocopherols (mg/kg)	42.5[a]	5.3	23.4[b]	4.8
α-Tocopherol (mg/kg)	0		0	
β-Tocopherol (mg/kg)	11.1	3.6	7.7	0.4
γ-Tocopherol (mg/kg)	10.1[a]	1.3	3.4[b]	1.7
δ-Tocopherol (mg/kg)	21.3	4.8	12.3[b]	3.2

[a]Values with different superscript letters were significantly different at $P < 0.05$. For other abbreviation see Table 1.

are major factors determining its thermal oxidative stability. In this study, TAG samples had the same FA composition, but the thermal oxidative stability was different among TAG samples.

The effect of TAG molecular species on thermal oxidative stability was was thought to be due to different rates of radical propagation and termination during thermal oxidation. In a review by Frankel (15), he indicated that thermal oxidation is a free-radical chain reaction that produces hydroperoxides, which are then converted to secondary oxidation products such as carbonyl compounds, FFA, and polymerized compounds. Free-radical chain reactions are well known to consist of three stages: initiation, propagation, and termination. In this study, the FA composition of the TAG samples was the same, so the initiation rate was also the same. Thus, the propagation and termination rates might be related to differences in the thermal oxidative stability of the TAG samples.

For example, 18:2 is a major factor determining the oxidative stability of TAG samples containing 16:0 and 18:2 (2:1). In PPP/LLL (2:1), 18:2 is located closely to another 18:2 in a single TAG molecule. A peroxyl radical produced on 18:2 can easily abstract the hydrogen from another 18:2 located in the same TAG molecule to form a hydroperoxide. Similarly, a peroxyl or alkyl radical produced on 18:2 in PPP/LLL (2:1) may easily react with another peroxyl or alkyl radical to produce polar monomers and polymers. Actually, the content of polar monomers and polymers was higher in PPP/LLL (2:1) after heating (Table 1). On the other hand, it may be not easy for a peroxyl radical produced on 18:2 in PPL to abstract the hydrogen from another 18:2, because the 18:2 in PPL is located farther away from 18:2 than in the LLL molecule. Thus, PPP/LLL (2:1) showed lower thermal oxidative stability, because it underwent the quick propagation and termination of a free-radical chain reaction.

Pongracz (16) reported that the thermal stability of tocopherol depended on the unsaturation of edible oils, and that it was higher in unsaturated oils. However, our result was different from that result. The thermal stability of tocopherol in TAG samples was affected by the TAG molecular species, even though the unsaturation of TAG samples was the same. Tocopherol was unstable in TAG samples containing highly unsaturated TAG molecular species (OOO, LLL, and LnLnLn). TAG structure may affect the nutritional value of frying and heated oils.

Thus, TAG structure, as well as FA composition and the presence and identity of an antioxidant, may determine the thermal oxidative stability of edible oils.

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