Effect of Fatty Acid Positional Distribution and Triacylglycerol Composition on Lipid By-Products Formation During Heat Treatment: I. Polymer Formation

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ABSTRACT: Effects of the fatty acid positional distribution and of the triacylglycerol (TG) composition on polymerization of TG during heat treatment were studied. Diacid TG molecules, acylated only with linoleic acid or linolenic acid along with palmitic acid, and positioned either in the central position (PLP and PLnP, respectively) or in one of the two outer positions (PPL and PPLn, respectively) were synthesized. Monoacid TG, i.e., trilinolein and trilinolenin, were also synthesized and mixed with tripalmitin in a 1:2 ratio. These model TG were also compared to TG models that consisted of a canola oil and its randomized counterpart, whose fatty acid positional distribution and TG composition were determined by means of high-performance liquid chromatography (HPLC). After heating, the polymer content and composition were evaluated by HPLC-size exclusion chromatography. Both pure TG and the canola oil models showed that acylation of polyunsaturated acids in the central position was protective against polymerization, although the effect was mainly observed with linolenic acid. The synthetic-TG study showed that the monoacid TG species exhibited higher sensitivity toward polymerization than the diacid species. The slight differences in the TG species between both canola oils did not allow observation of such a relationship with regard to TG composition.
JAOCS 75, 1065–1071 (1998).

KEY WORDS: Canola oil, heat treatment, interesterified oil, TG composition, TG structure, polymer.

The effects of structure and composition of triacylglycerols (TG) on their stability to oxidation have been long investigated, but variable results have been obtained. In some studies, randomization or interesterification of vegetable oils has been reported to decrease their stability (1–3). In contrast, other authors have not found significant differences in oxidative changes, either at low (4) or high temperature (5). Also, when native and randomized vegetable oils were tested at 28 and 55°C, no differences were found at 55°C, while randomized oils featured decreased stability at 28°C (6).

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Similarly, studies with oils and model systems have been carried out to define whether the unsaturated fatty acyl group located in the 2-position of the TG molecule was protected against oxidation. In assays wherein oils were heated at high temperatures (7,8), results led to the conclusion that unsaturated fatty acids were lost to a proportionally greater extent from the TG outer positions (*sn*-1/3) than from the central position. These observations agreed with data obtained by Wada and Koizumi (8) in model systems at low temperatures but were not consistent with those obtained by Park *et al*. (4).

In the present study, special attention has been placed on the formation of polymeric compounds in systems that differed in TG structure and composition. Polymers constitute the primary group of new compounds formed in fats that are heated at high temperature in processes such as frying, which is presently an important source of consumption of high amounts of fats and oils (9). Formation of polymers clearly depends on temperature (10–12), surface-to-volume of oil ratio (12), period of heating (10,13), and fatty acid composition (10,14,15), among other variables. With respect to TG composition, it is known that the most unsaturated TG species are lost preferentially during heating (14,16). Nevertheless, it remains unclear how TG structure and TG molecular species composition might modulate the formation of polymers. To address this issue, different model TG systems with identical fatty acid compositions but differing in the position of the unsaturated fatty acyl group or in the molecular species composition were synthesized. The susceptibility to polymer formation of more natural models, a canola oil and its randomized counterpart, also differing in TG structure and TG composition, was also determined. To simplify the interpretation of data, model TG were heated in the absence of air, thus promoting mainly C–C linkages and thereby limiting the complexity of the newly formed compounds.

EXPERIMENTAL PROCEDURES

Pure TG. 1,2-Dipalmitoyl-3-linolein (PPL), 1,2-dipalmitoyl-3-linolenin (PPLn), 1,3-dipalmitoyl-2-linolein (PLP), 1,3-dipalmitoyl-2-linolenin (PLnP), and mixtures of trilinolein

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(LLL) and tripalmitin (PPP) (1:2) and of trilinolenin (LnLnLn) and PPP (1:2) were used. In all model systems, the molar ratio of saturated-to-unsaturated fatty acids was 2:1. Syntheses of TG are described later.

Oils. Purified TG from canola oils were interesterified at 75°C with sodium methoxide (0.2 % wt/vol) for 3 h with continuous stirring. The resulting randomized TG were washed once with 0.5 N sodium hydroxide, and twice with a 3% sodium chloride solution. Both the randomized and the original oil TG were then heated as described below.

Heat treatment. Glass ampoules with 100 mg of pure TG or oil samples were sealed under vacuum and heated at 180, 200, 220, and 240°C in an oven for 15 h (canola oils and model TG with 18:3n-3) or 30 h (model TG with 18:2n-6). Lipids were extracted with chloroform/methanol (2:1, vol/vol), and the solvent was reduced to a minimum with nitrogen for storage at −20°C prior to analysis.

Analysis of dimers and higher oligomers of TG. Dimers and higher oligomers of TG were quantitated by high-performance size-exclusion chromatography (HPSEC) by following the IUPAC Standard Method (17) with slight modifications. Briefly, 10 µL of sample solution (50–100 mg/mL tetrahydrofuran) was injected in a Konik 500A chromatograph (Konik, Barcelona, Spain) with a 10-µL sample loop. A refractive index detector (Hewlett-Packard, Pittsburgh, PA), one 100Å Ultrastyragel, and one 500 Å Ultrastyragel column (Waters Associates, Milford, MA), connected in series, were operated at 35°C. The columns were 25 × 0.77 cm i.d., packed with a porous, highly cross-linked styrene-divinylbenzene copolymer (<10 mm). HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. Three groups of compounds were separated according to differences in molecular size: monomers of TG, dimers of TG, and higher oligomers of TG. Quantitation of dimers and oligomers was based on individual peak areas, assuming equal response factors.

Analysis of dimers of fatty acids as methyl esters (FAME). Samples from each treatment were pooled, and 50 mg was transmethylated with potassium hydroxide in approximately 2 N methanolic solution (18). Dimers of FAME were quantitated in samples of *ca.* 100 mg/mL by means of the methodology described previously for dimers and oligomers.

Polar compound determination. Polar compound determination was carried out with 50 mg of the pooled samples, by a combination of solid-phase extraction and HPSEC, with monostearin as internal standard. The methodology has been described in detail, including precision, accuracy, and recovery data, in a recent publication (19). Briefly, 2 mL of sample solution, containing 50 mg of sample and 1 mg monostearin, was placed on the 1-g silica cartridge. The nonpolar fraction was eluted with 15 mL petroleum ether/diethyl ether (90:10, vol/vol), and the polar fraction, containing polar compounds and internal standard, with 15 mL diethyl ether. After evaporation, the polar fraction was redissolved in tetrahydrofuran for analysis by HPSEC, under the same conditions applied above.

TG synthesis. The preparation of pure model TG was carried out as described previously (20). Briefly, monoacid triacyl-*sn*-glycerols were synthesized with 1 mol of glycerol esterified with 3 mol of free fatty acids (either 16:0, 18:2n-6, or 18:3n-3) solubilized in CH_2Cl_2, and 3.3 mol of 4-dimethylaminopyridine (DMAP) in the presence of 1.1′-dicyclohexylcarbodiimide (DCC) as presented elsewhere (21). TG were purified by silicic acid dry-column chromatography as described (20) and adapted from (22) with a mixture of hexane/diethyl ether (80:20, vol/vol). The resulting trilinolein (LLL) and trilinolenin (LnLnLn) were mixed with tripalmitin (PPP) in a 1:2 ratio. Diacid triacyl-*sn*-glycerol with fatty acids at defined positions on the glycerol backbone was prepared from diacylglycerol (DG) as starting material. For TG with polyunsaturated fatty acids (PUFA) located in the central position, 1,3-dipalmitoyl-dihydroxypropan-2-one was used and prepared with 1 mol of dihydroxyacetone and 2 mol of palmitic acid as previously described (20), and adapted from the method of Bentley *et al.* (23). The resulting 1,3-dipalmitoyl-dihydroxypropan-2-one was purified by crystallization from hot methanol and reduced to 1,3-dipalmitoyl-*sn*-glycerol with $NaBH_4$. The 1,3-diacyl-*sn*-glycerol was purified by borate-impregnated silicic acid dry-column chromatography (20) by an adapted procedure (22) with $CHCl_3$/acetone (96:4, vol/vol) as developing solvent. The synthesis of TG with PUFA in one of the two outer positions started with 1,2-dipalmitoyl-*rac*-glycerol. These DG were prepared as described elsewhere (20); isopropylidene-*O*-benzyl-*rac*-glycerol was synthesised from isopropylidene-*rac*-glycerol and chloromethylbenzene (24). This was then hydrolyzed (24) to yield 3-*O*-benzyl-*rac*-glycerol. The 3-*O*-benzyl-*rac*-glycerol was then acylated with palmitic acid by using DMAP and DCC in CH_2Cl_2 as described above for the dihydroxyacetone. The resulting 3-*O*-benzyl-1,2-dipalmitoyl-*rac*-glycerol was purified by crystallization from hot methanol and was then debenzylated as described elsewhere (20,25). The resulting 1,2-dipalmitoyl-*rac*-glycerol was purified from the unreacted materials and the trace amounts of isomerized 1,3-dipalmitoyl-*sn*-glycerol by silicic acid dry-column chromatography impregnated with boric acid as described above for the 1,3-diacyl-*sn*-glycerol, except that hexane/diethyl ether/acetic acid (60:40:4, vol/vol/vol) was used as the developing solvent. The PUFA were acylated in the *sn*-2 position of the 1,3-dipalmitoyl-*sn*-glycerol or in the *sn*-1 or -3 position of the 1,2-dipalmitoyl-*rac*-glycerol by using DMAP and DCC, and the resulting triacyl-*sn*-glycerols were purified by dry-column chromatography as described above. The composition of the synthesized TG with linoleate or linolenate either in the *sn*-2 (PLP and PLnP, respectively) or in one of the two outer positions (PPL and PPLn, respectively) was ascertained by analysis of the fatty acid composition of the 2-monoacyl-*sn*-glycerol released after pancreatic lipase lipolysis (26,27).

Stereospecific analysis. The fatty acids positional distribution in the purified TG from the canola oils was determined as previously published (28,29). The TG were first partially deacylated with ethyl magnesium bromide, and the reaction

products were fractionated by thin-layer chromatography (TLC) with chloroform/acetone (96:4, vol/vol). All bands that corresponded to acylglycerols and an aliquot of the *rac*-diacylglycerol were scraped off from the plate and transmethylated with BF_3/methanol (14%). The remaining diacyl-*rac*-glycerol was processed for fractionation by chiral-phase HPLC after derivatization into dinitrophenyl urethanes with 3,5-dinitrophenyl isocyanate (28,29). The resolution of the enantiomeric *sn*-1,2- and *sn*-2,3-DAG as their 3,5-dinitrophenylurethane derivatives was performed by HPLC with a Spectra Physics SP 8810 (San Jose, CA) isocratic pump and a chiral column (25 cm × 4.6 mm i.d.) of (R)-(+)-1-(1-naphthyl) ethylamine polymeric phase covalently bonded to 300 Å wide-pore spherical silica particles (5 µm, YMC-Pack A-K03; YMC Inc., Kyoto, Japan). The separation was done isocratically at 8°C with hexane/1,2-dichloroethane/ethanol (40:10:1, vol/vol/vol) as mobile phase at a constant rate of 0.5 mL/min. Peaks were monitored at 254 nm with a Uvikon 730-LC UV-spectrophotometer (Kontron AG, Zurich, Switzerland). The TG structure was calculated from the fatty acid composition of the DG enantiomers, as well as from the fatty acid composition of the 2-monoacyl-*sn*-glycerol obtained by Grignard degradation and pancreatic lipase hydrolysis (29).

TG molecular species analysis. Purified TG from the canola oils were fractionated by reversed-phase HPLC on a column of ChromSpher™ C_{18} (3 mm particles; 200 × 4.6 mm i.d.), with gradient elution from acetone/acetonitrile 60:40 (vol/vol) to 70:30 over 60 min at a flow rate of 0.6 mL/min. A Hitachi (Tokyo, Japan) L-6200A HPLC pump was used with a Varex Model III evaporative light-scattering detector (P.S. Instruments, Sevenoaks, United Kingdom).

Statistical evaluation. Significance of the differences between the means found for total polymers of TG was analyzed by analysis of variance on ranks. Student's Neuman Keuls test was used as soon as heterogeneity between groups was demonstrated. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Pure model TG study. Tables 1 and 2 show the values of dimers, higher oligomers, and total polymers of TG obtained for synthetic TG that contained 18:2n-6 and 18:3n-3, respectively. Given the great importance of controlling the variables involved in assays at high temperature, samples heated to the same temperature were prepared simultaneously.

The presence of oxygenated compounds could be clearly detected in some samples. Heating at high temperature in the absence of air does not modify color appreciably. Reactions are slow, and monomeric and polymeric compounds without extra oxygen are formed, i.e., TG contain cyclic and isomeric fatty acids, nonpolar radical or Diels-Alder dimers of TG, etc., and the range of polarity of the new compounds is limited. In contrast, the presence of oxygen greatly accelerates reactions, giving rise to a high number of new oxygenated structures with a wide range of polarity, and samples darken easily (30). Thus, simply by checking the color and polarity range by TLC, the presence of oxygenated compounds in some of the samples tested was clearly detected. This was possibly due to failures in the sealing of ampoules. Only samples wherein the absence of oxygen could be guaranteed were evaluated and included in statistical analyses. Otherwise, data are not presented.

Results showed different behavior for diacid systems with 18:2n-6 and 18:3n-3 in different positions on the TG molecule. Although slightly higher values were generally found for PPL than for PLP (Table 1), overall differences were not significant. In contrast, for 18:3n-3 (Table 2), differences between PPLn and PLnP were significant at the four temperatures. Thus, from the overall results, it seems that the 2-position of the TG molecule showed a protective effect that is more evident in linolenic acid systems.

When comparing monoacid and diacid TG, the main difference was that polymerization seemed to take place to a

TABLE 1
Dimer, Higher Oligomer, and Total Polymer Contents (wt%) Formed from Pure Model Triacylglycerols (TG) Acylated with Linoleic Acid and Heated for 30 h

	Oligomers[a]			Dimers[b]			Total[c]		
Temperature (°C)	PLP	PPL	PPP/LLL (2:1)	PLP	PPL	PPP/LLL (2:1)	PLP	PPL	PPP/LLL (2:1)
180	n.d.[d]	n.d.	n.d.	0.6 (0.10)	0.7 (0.01)	0.7 (0.06)	0.6 (0.1)	0.7 (0.01)	0.8 (0.14)
200	n.d.	n.d.	0.2[a] (0.02)	0.8 (0.01)	0.8 (0.06)	1.0 (0.04)	0.8 (0.01)	0.8 (0.06)	1.2 (0.03)
220	0.2 (0.03)	0.2 (0.01)	0.2 (0.03)	1.7[a] (0.08)	2.7[b] (0.08)	2.6[b] (0.18)	1.9[a] (0.09)	2.9[b] (0.10)	2.8[b] (0.20)
240	0.3[a] (0.04)	0.4[a] (0.10)	0.7[b] (0.05)	3.5 (0.24)	3.5 (0.22)	3.6 (0.12)	3.8 (0.28)	3.8 (0.16)	4.2 (0.17)

[a]Oligomers of TG higher than dimers.
[b]Dimers of TG.
[c]Sum of oligomers and dimers.
[d]n.d., not detectable; numbers in parentheses are SEM (n = 2 to 3); for each compound, results with different superscript letters in the same row are statistically different ($P < 0.05$). PLP, 1,3-dipalmitoyl-2-linolein; PPL, 1,2-dipalmitoyl-3-linolein; PPP/LLL, tripalmitin/trilinolein.

TABLE 2
Dimer, Higher Oligomer, and Total Polymer Contents Formed from Pure Model TG Acylated with Linolenic Acid and Heated for 15 h

Temperature (C)	Oligomers[a]			Dimers[b]			Total[c]		
	PLnP	PPLn	PPP/LnLnLn (2:1)	PLnP	PPLn	PPP/LnLnLn (2:1)	PLnP	PPLn	PPP/LnLnLn (2:1)
180	n.d.	n.d.	n.d.	0.2[a] (0.02)[d]	0.5[b] (0.03)	0.5[b] (0.05)	0.2[a] (0.02)	0.5[b] (0.03)	0.5[b] (0.05)
200	n.d.	n.d.	0.1[a] (0.00)	0.2[a] (0.01)	0.7[b] (0.02)	0.8[b] (0.05)	0.2[a] (0.01)	0.7[b] (0.02)	0.9[c] (0.05)
220	n.d.	0.7[a] (0.07)	0.4[a] (0.12)	1.0[a] (0.03)	4.0[b] (0.19)	2.1[c] (0.19)	1.0[a] (0.03)	4.7[b] (0.27)	2.6[c] (0.32)
240	0.7[a] (0.08)	1.1[a] (0.10)	2.4[b] (0.22)	4.3[a] (0.21)	5.7[b] (0.10)	4.9[a] (0.15)	5.0[a] (0.29)	6.8[b] (0.20)	7.3[b] (0.07)

[a]Oligomers of TG higher than dimers.
[b]Dimers of TG.
[c]Sum of oligomers and dimers.
[d]n.d., not detectable; numbers in parentheses are SEM (n = 2 to 3); for each compound, results with different superscript letters in the same row are statistically different ($P < 0.05$). PLnP, 1,3-dipalmitoyl-2-linolenin; PPP/LnLnLn, tripalmitin/trilinolenin; PPLn, 1,2-dipalmitoyl-3-linolenin. See Table 1 for other abbreviation.

lower level of dimers in the monoacid TG systems (LLL/PPP and LnLnLn/PPP). This is clearly observed in samples with the greatest content of total polymers (those heated at 240°C), which show much higher values of oligomers for monoacid than for diacid TG. For instance, in systems with 18:2, all with a similar amount of dimers, the oligomer content was double for LLL/PPP (1:2) (Table 1). Additionally, in 18:3n-3 systems, values found for dimers of LnLnLn/PPP (1:2) were intermediate between those of PLnP and PPLn, but at the highest temperature, the oligomer content for LnLnLn/PPP (1:2) was significantly higher (2.4%) than that for PPLn (1.1%) and PLnP (0.7%). Also, total polymers showed a significant increase beyond 200°C, in agreement with the results reported previously on thermal oxidation (12). Furthermore, total polar compounds were quantitated to determine the relevance of total polymers among the newly formed compounds of higher polarity than the starting TG. Results are presented in Table 3 and, as expected, the values were higher than those corresponding to total polymeric compounds, shown in Tables 1 and 2. Clearly, polymers of TG were the most abundant group of compounds. A linear regression was obtained (wt% polymers = 0.84 × wt% polar compounds – 0.43), with a high regression coefficient (0.978) and values for the standard errors for regression, intercept, and slope of 0.462, 0.159, and 0.039, respectively. Taking into account that both polymers and polar compounds were expressed as wt% of fat, the value of the slope indicates a high average ratio. It is interesting that TG molecules, acylated with one PUFA in the 2-position (PLP and PLnP), always gave rise to lower amounts of polar compounds than those TG that are acylated with one PUFA in one of the two outer positions (PPL and PPLn), thus confirming a protective effect of the central position.

TABLE 3
Quantitation of Polar Compounds (wt%)[a]

Temperature (°C)	PLP	PPL	PPP/LLL (2:1)	PLnP	PPLn	PPP/LnLnLn (2:1)
180	1.1	1.8	1.6	0.5	1.0	0.8
200	1.3	1.9	1.9	0.6	1.4	1.2
220	2.9	—	3.8	2.4	6.2	5.1
240	4.5	4.9	5.3	6.7	7.1	9.5

[a]Pure model TG with linoleic acid were heated for 30 h, and those with linolenic acid were heated for 15 h. See Tables 1 and 2 for abbreviations.

One of the advantages offered by the model systems selected is the opportunity to analyze intradimer formation in the systems that contain monoacid TG. Formation of intramolecular dimers, i.e., linkage of two unsaturated fatty acids belonging to the same TG molecule, would be possible only in systems that contain monoacid TG, while only intermolecular dimers (involving two TG molecules) could be formed in the diacid systems. Hence, in monoacid systems, the FAME dimers obtained after transesterification would result both from the intermolecular linkages (involving two different molecules of TG) and from the intramolecular linkages (involving PUFA belonging to the same TG molecule), whereas they were formed only by intermolecular linkages in diacid systems. It is clear that, for similar amounts of total polymers, the existence of intramolecular dimerization in diacid systems would give rise to much higher amounts of dimers of FAME after transesterification than those obtained exclusively by intermolecular dimerization (31,32).

Table 4 summarizes the results for dimers of FAME after transesterification of the samples pooled for each system and temperature. Given the differences that existed in total polymers between samples, dimers of FAME-to-total polymers of TG ratios (values between brackets) better reflected the results obtained. As already noted, according to their structure, the presence of intradimers in appreciable amounts would give much higher ratios in monoacid TG than in diacid TG. However, the values obtained were of the same magnitude in both systems, even at the highest temperature, thus indicating that intradimerization did not occur to an appreciable extent. Finally, oligomers of FAME of molecular weight higher than dimers of FAME were only detected in trace amounts in

TABLE 4
Quantitation of Dimeric Fatty Acid Methyl Esters (%)[a]

Temperature (°C)	PLP	PPL	PPP/LLL (2:1)	PLnP	PPLn	PPP/LnLnLn (2:1)
180	0.3 (0.5)[b]	0.3 (0.43)	0.5 (0.62)	trace	0.2 (0.40)	0.2 (0.40)
200	0.4 (0.50)	0.4 (0.50)	0.6 (0.50)	trace	0.4 (0.57)	0.5 (0.57)
220	0.7 (0.37)	—	1.2 (0.43)	0.4 (0.40)	1.8 (0.38)	1.3 (0.50)
240	1.6 (0.42)	1.7 (0.44)	2.0 (0.47)	2.1 (0.42)	2.8 (0.41)	3.3 (0.45)

[a]Pure model TG with linolenic acid were heated for 15 h, and those with linoleic acid were heated for 30 h.
[b]Numbers in parentheses are dimers of fatty acid methyl esters-to-total polymers of triacylglycerol ratios. See Table 1 for abbreviations.

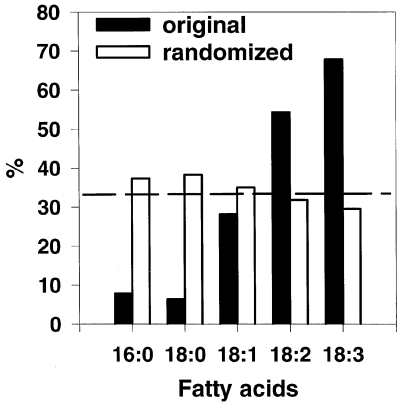

FIG. 1. Fatty acid composition in the *sn*-2 position of the original canola oil and of the interesterified oil as determined by pancreatic lipase hydrolysis. The dashed line set at 33% indicates a theoretical random distribution.

LnLnLn samples, supporting the suggestion that total polymers of TG were primarily formed through dimeric linkages.

Canola oils. Table 5 reports the fatty acid composition and TG structure of the original canola oil. It shows that the saturated fatty acids were acylated mainly in the *sn*-3 position, to a lesser extent in the *sn*-1, and least in the *sn*-2 position. Among the monoenes, 18:1n-9 was almost equally distributed in the three *sn*-positions, whereas 18:1n-7 was predominantly esterified in the *sn*-3 and *sn*-1 positions. The positional distribution of both 18:2n-6 and 18:3n-3 was similar, with a preferential localization in the *sn*-2 position. However, as observed in Figure 1, the fatty acids were evenly distributed in the three positions of the *sn*-glycerol for the randomized oil. Table 6 presents the TG compositions of both the original and the randomized oils. The TG molecular species were rather similar in the original and the randomized oils. For instance, more than 50% of the TG species in both oils was made up of both triolein (one-third) and dioleoyllinoleoyl-*sn*-glycerol (one-fourth). Trilinolein also accounted for 5.3% of the TG, while no trilinolenin was detected. However, slight differences were observed. For example, PUFA are generally more evenly distributed among the TG species of the randomized oil than in the original oil. This is best illustrated by the unsaturation index, which is higher for the original (0.47) than for the randomized oil (0.36).

The results found with the pure model TG were also confirmed to some extent with both canola oils. The PUFA in the original canola oil are mainly located in the central position, compared to the randomized oil (Fig. 1), and consistent with the observation that, between PLnP and PPLn, the former is also more resistant to polymer formation, although the difference reached statistical significance only at 220°C (Table 7). On the other hand, at any temperature, the original oil with the highest content of unsaturated TG never formed more dimers and oligomers than the randomized oils in which the PUFA are more evenly distributed within the TG species (Table 6). This is in contradiction with the results obtained with the model TG, for which the most unsaturated TG species (LLL and LnLnLn) generally formed more polymers than the less unsaturated molecules (PPL, PPLn, PLP, and PLnP). Besides

TABLE 5
Fatty Acid Positional Distribution in the TG of the Original Canola Oil

Fatty acids	*sn*-1[a] (%)	*sn*-2[a] (%)	*sn*-3[a] (%)	TG (%)
16:0	39.2	4.0	56.8	8.0
18:0	36.7	2.9	60.4	4.9
18:1n-9	35.1	32.5	32.4	59.0
18:1n-7	49.5	11.0	39.6	2.8
18:2n-6	24.3	53.7	22.0	18.7
18:3n-3	24.7	55.0	20.4	6.3

[a]Distribution (%) of single fatty acids among the three *sn* positions. See Table 1 for abbreviation.

TABLE 6
TG Composition of the Original and of the Randomized Canola Oils

TG species[a]	Original oil	Randomized oil
LLLn	1.0	nd
OLnLn	1.1	0.6
LOLn	1.1	0.7
LLL	5.3	5.3
LPLn	0.4	0.3
LLO	8.5	7.5
LnOO	8.9	8.7
LLP	1.0	0.3
LnOP	1.2	1.1
LOO	24.3	26.4
LOP	3.6	3.0
SLnP	1.2	1.2
OOO	33.0	33.7
OOP	5.6	6.3
OPP	1.7	1.9
OOS	2.2	2.3
Unsaturation index[b]	0.47	0.36

[a]L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; S, stearic acid. The letter order does not indicate the stereospecific distribution.
[b]Calculated as the ratio of [TG with at least two polyunsaturated fatty acids (PUFA)]/(TG with only one PUFA) × 100. See Table 1 for abbreviation.

TABLE 7
Dimer, Higher Oligomer, and Total Polymer Contents Formed from the Original or the Randomized Canola Oil TG Heated for 15 h[a]

Temperature	Oligomers[b]		Dimers[c]		Total[d]	
(°C)	Original	Randomized	Original	Randomized	Original	Randomized
180	0.1 (0.01)	1.1 (0.01)	1.1 (0.01)	1.1 (0.09)	1.2 (0.02)	1.2 (0.09)
200	0.1 (0.02)	0.1 (0.03)	1.1 (0.04)	1.1 (0.15)	1.3 (0.06)	1.3 (0.18)
220	0.2 (0.02)	0.4[e] (0.01)	1.7 (0.03)	2.1[e] (0.04)	1.8 (0.04)	2.5[e] (0.03)
240	0.4 (0.01)	0.4 (0.05)	2.8 (0.05)	3.0 (0.11)	3.2 (0.06)	3.4 (0.17)

[a]Results are expressed as mean ± SEM (n = 2 to 3).
[b]Oligomers of TG higher than dimer.
[c]Dimer of TG.
[d]Sum of oligomers and dimers.
[e]Statistically different from the original oil ($P < 0.05$). See Table 1 for abbreviation.

18:2n-6 and 18:3n-3, and in contast to the pure model TG, both canola oils contained high amounts of oleic acid, which is able to polymerize (33). The unsaturated fatty acids are combined in TG in several ways, generating TG species that contain 1, 2, or 3 PUFA, for instance. This higher level of complexity certainly obscured the conclusion drawn with the pure model TG. Taken together, our findings would indicate that the slight differences in the TG composition between the original and the randomized oils are not great enough to influence the polymerization rate and that, under our experimental conditions, the TG structure is a more important factor to explain the differences in polymer contents.

In conclusion, our study showed the effects of both positional distribution of PUFA and of TG molecular species on thermal polymerization. The effect of the positional distribution was observed mainly in systems that contained linolenic acid, whereas the effect of the TG composition was noticed in TG systems with 18:3n-3 as well as 18:2n-6. The differences found with a more realistic model, consisting in a natural oil or its interesterified counterpart, support an effect of the fatty acid positional distribution. Similar studies are required to examine the influence of both TG structure and composition on polymerization under conditions of heating in air.

ACKNOWLEDGMENTS

This work was funded in part by the Scottish Office Agriculture, Environment and Fisheries Dept. and by CICYT (Project ALT 95-0736).

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[Received September 8, 1997; accepted April 20, 1998]