Effect of Fatty Acid Positional Distribution and Triacylglycerol Composition on Lipid By-Products Formation During Heat Treatment: III—Cyclic Fatty Acid Monomers Study

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ABSTRACT: The influence of isomeric triacylglycerols (TG) containing 18:3n-3 and 18:2n-6 on the formation of cyclic fatty acid monomers (CFAM) after heat treatment was assessed. Diacid TG, containing linoleic acid (L) or linolenic acid (α-Ln) along with palmitic acid (P), 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 of trilinolein and trilinolenin mixed with tripalmitin were also prepared. The CFAM formed after heating were analyzed after total hydrogenation. The results obtained with the model TG were compared to another model consisting of a canola oil and its randomized counterpart. In diacid TG, the location of α-Ln in the central position of the TG molecule (PLnP) greatly enhanced the formation of the CFAM upon heating at temperatures below 240°C. On the other hand, 18:3n-3 in monoacid TG (LnLnLn) was highly resistant to CFAM formation within the same range of temperatures (180–220°C). The TG structure, more than the TG composition, seemed to explain the differences in the CFAM formation between the original canola oil and its interesterified counterpart. Like α-Ln, 18:2n-6 was more prone to cyclization when attached at the central position of the model TG. Conversely, the influence of the TG composition on the cyclization rate was less important for L than for α-Ln. It was concluded that positioning the C18 polyunsaturated fatty acid in the central position of TG rendered the oils much more sensitive to the cyclization reaction upon heat treatment.

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KEY WORDS: Canola oil, cyclic fatty acid monomers, heat treatment, hydrogenated fatty acids, interesterified oil, TG composition, TG structure.

Many chemical reactions like oxidation, polymerization, hydrolysis, isomerization, and cyclization occur during deep-fat frying or industrial refining of oil (1). Some of these reactions lead to the formation of cyclic fatty acid monomers (CFAM), mainly from 18:3n-3 and 18:2n-6 (2–4). When fed to rats as a purified fraction, these CFAM are incorporated into cellular lipids (4), and those formed from linolenic acid (Ln) have been associated with several adverse effects such as poor growth and high mortality after birth (4), alteration of electrophysiological parameters as well as resistance to reoxygenation after hypoxia of heart cells in culture (5), and modification of some enzymatic activity in liver (6,7), intestine (8), or cultured endothelial cells (9).

Heating times and temperatures as well as the relative content of 18:2n-6 and 18:3n-3 fatty acids in the oils influence CFAM formation (10). The cyclization of CFAM is assumed to proceed *via* a radical-mediated mechanism (11). This type of mechanism is also involved in the autoxidation of polyunsaturated oils (12) and likely in the geometrical isomerization of polyunsaturated fatty acids (PUFA) (13). Numerous studies have dealt with the influence of triacylglycerol (TG) structure upon the oxidative stability of their constituent fatty acids (14–17). Although these studies gave conflicting results with regard to the structure allowing more or less autoxidation sensitivity, the importance of this structural factor to the sensitivity of a given oil to oxidation has been recognized. Moreover, not only the stereospecific distribution of the PUFA within the TG molecule may affect their oxidative stability but also the molecular species in the test oils (17). Additionally, we recently determined that both the TG structure and the TG fatty acid composition may modulate the formation of *trans*-isomers of α-Ln whereas only the TG composition was able to influence the formation of *trans*-isomers from linoleic acid (L) (18).

Based on the observations regarding the radical-mediated mechanisms underlying thermal autoxidation, geometrical isomerization, and presumably cyclization of PUFA, we designed an experiment that used simple model TG with defined structures, and a native canola oil with its randomized counterpart, to address whether a TG factor can also modulate CFAM formation.

EXPERIMENTAL PROCEDURES

Samples and heat treatment. 1,2-Dipalmitoyl-3-linolein (PPL), 1,2-dipalmitoyl-3-linolenin (PPLn), 1,3-dipalmitoyl-2-linolein (PLP), 1,3-dipalmitoyl-2-linolenin (PLnP), a mix-

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ture of trilinolein (LLL) and tripalmitin (PPP) (1:2) and of trilinolenin (LnLnLn) and PPP (1:2) were synthesized as described (24,25). In all model systems, the molar ratio of saturated to unsaturated fatty acids was 2. Canola oils (natural and interesterified) were identical to those used in Parts I and II of this series of reports (18,19). They were composed of C16:0 (8.0%), C18:0 (4.9%), C18:1Δ9*c* (59.0%), C18:1Δ11*c* (2.8%), C18:2Δ9*c*,12*c* (18.7%), and C18:3Δ9*c*,12*c*,15*c* (6.3%).

Structural analysis and TG composition analysis. The high-performance liquid chromatography (HPLC) methods used to carry out both the stereospecific and TG molecular species analyses of the canola oils have been detailed by Martin *et al.* (19).

Heat treatment and analysis of the CFAM. Glass ampoules containing 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 containing Ln) or 30 h (model TG containing L). Each TG sample was run in triplicate. Lipids were extracted with chloroform/methanol (2:1, vol/vol), and the solvent was reduced to a minimum under a stream of nitrogen for storage at –20°C prior to analysis. An aliquot (20 to 40 mg) of each of the heated TG was transesterified with boron trifluoride in methanol (14%, vol/vol) as described (20). The resulting fatty acid methyl esters (FAME) were extracted with 2 × 2 mL of hexane and 2 mL of water and dried over Na_2SO_4. A known amount of 16:0 ethyl ester was added to the FAME, and the mixture was then hydrogenated on platinum oxide (21). The resulting hydrogenated FAME were fractionated by HPLC on a C18 reversed-phase column (Shandon, Cergy-Pontoise, France) (Ultrabase, 250 mm length, 10 mm i.d., 5 µm particle diameter), using acetonitrile/acetone (90:10, vol/vol) at 4 mL/min (21). The fraction containing the CFAM and the internal standard was further analyzed by gas–liquid chromatography (GLC) on a polar capillary column. FAME analysis from the TG was carried out on a Hewlett-Packard gas chromatograph (model 5890; Les Ulis, France) fitted with a flame-ionization detector and a split-splitless injector, both set at 250°C. The carrier gas was helium (1.1 mL/min), and elution was performed with a BPX 70 column (SGE, Villeneuve-Saint-Georges, France) (50 m length, 0.33 mm i.d., and 0.25 µm film thickness). The column was operated at 60°C for 1.1 min and the temperature was raised to 170°C at a rate of 20°C/min, held for 20 min, then increased at 10°C/min to 220°C and held at this temperature for 5 min. Quantitation was made by peak integration performed with a Spectra-Physics Chromjet integrator and a Spectra-Physics Chemstation operated by the WOW software (Spectra-Physics, La Verpillière, France). CFAM occurring from α-Ln were identified using a standard mixture prepared from a heated linseed oil whose detailed analysis had already been performed (21), and those occurring from L from a standard mixture prepared from a sunflower oil (21). Additionally, confirmation of the structure of the CFAM arising from L was achieved by gas chromatography–mass spectrometry (GC–MS) using dimethyloxazoline (DMOX) derivatives.

Preparation of the DMOX derivatives and GC–MS analysis. FAME (500 µg, dried under nitrogen in an ampoule) were converted to the DMOX derivatives with 500 µL 2-amino-2-methylpropanol (22). The reaction was allowed to proceed at 170°C for 18 h at 180°C. After heating, the DMOX were extracted twice with 2 mL dichloromethane after addition of 2 mL distilled water. The dichloromethane phase was washed with distilled water to neutral pH.

GC–MS analyses of DMOX derivatives were carried out on a Hewlett-Packard MSD 5970 B quadrupole mass spectrometer with an ion source of 70 eV, fitted with a Hewlett-Packard 5890 II model chromatograph. The DMOX derivatives were eluted on a BPX 70 column.

Statistics. Results were computed with Microsoft Excel and statistics operated with SigmaStat® (Jandel Scientific, San Rafael, CA). Results were expressed as mean ± SEM (n = 3 for each sample heated). For multiple comparisons (pure model TG groups), significance of the differences between the means found for the isomers was analyzed by the analysis of variance on ranks. Student-Neuman-Keuls test was used as soon as heterogeneity between groups was demonstrated. The Mann and Whitney rank sum test was used when only two groups had to be compared (two canola oils). The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Although methods exist to analyze CFAM in their native form (11,23), we chose to analyze them fully hydrogenated. This method gives less information with regard to the structure, but it is suitable for quantitation purposes, because hydrogenation reduces the mixture to fewer molecular species (24). As a result, CFAM are more concentrated into fewer GLC peaks, which allows an improved confidence for quantitation, especially when dealing with temperatures where CFAM begin to form (e.g., 180 to 200°C).

As others have observed (2,10,25), the amount of CFAM formed was positively correlated with heating temperature in all of the experimental oils (Tables 1–3). Also, consistent with others (3,10), L is far less sensitive to cyclization, whatever the temperature, than α-Ln (50 times less in the present study, Tables 1 and 3), although heating time was greater for 18:2n-6 than for 18:3n-3 (30 vs. 15 h, respectively).

CFAM formed from α-Ln. Six main peaks detected by GLC corresponded to previously identified CFAM structures (26) (Fig. 1A): Peaks A, B, C, and E are five-membered ring CFAM, whereas peaks D and F are six-membered-ring CFAM. Statistical differences in the individual content of the CFAM species formed were found between the three model TG within the range of temperatures used. Nevertheless, none of the differences could be easily accounted for by an influence of the TG composition or structure, except that Ln in PPLn generally formed slightly more six-membered ring CFAM (e.g., compounds D and F) than in PLnP or LnLnLn (Table 1). Conversely, the acylation of Ln in the *sn*-2 position of the TG molecule resulted in greater formation of CFAM upon heat treat-

TABLE 1
Content (ppm) of Cyclic Fatty Acid Monomers[a] Formed from Linolenic Acid in Monoacid and Diacid Triacylglycerols Heated for 15 h at 180, 200, 220, and 240°C

	PPP/LnLnLn (2:1)[b]	PLnP[c]	PPLn[d]
180°C			
A	11.8 ± 0.3[a]	16.4 ± 0.2[b]	6.8 ± 1.1[c]
B	13.6 ± 0.6[a]	16.6 ± 0.3[a]	7.7 ± 2.0[b]
C	15.8 ± 1.2[a]	7.5 ± 1.0[b]	12.5 ± 1.3[a]
D	26.4 ± 1.5[a]	27.5 ± 0.1[a]	37.8 ± 3.0[b]
E	3.3 ± 0.1	7.5 ± 1.0	3.9 ± 0.8
F	29.3 ± 0.9	24.6 ± 0.5	31.4 ± 2.4
Total	21.4 ± 2.3[a]	81.5 ± 0.4[b]	59.0 ± 5.8[a]
200°C			
A	16.9 ± 2.9	21.7 ± 1.2	12.8 ± 1.1
B	15.3 ± 4.0	17.2 ± 1.2	12.3 ± 1.2
C	8.7 ± 0.6	7.0 ± 0.1	9.0 ± 1.2
D	28.3 ± 3.9[a]	21.7 ± 0.6[b]	32.1 ± 1.5[a]
E	3.7 ± 0.6[a]	8.8 ± 0.9[b]	4.2 ± 0.7[a]
F	27.1 ± 2.1	23.6 ± 0.2	29.5 ± 1.2
Total	111.0 ± 5.0[a]	218.6 ± 10.7[b]	135.0 ± 22.0[a]
220°C			
A	16.8 ± 0.0[a]	17.5 ± 0.2[a]	14.4 ± 0.6[b]
B	23.00 ± 0.0[a]	23.3 ± 0.2[b]	19.8 ± 1.3[a]
C	7.6 ± 0.2[a]	7.6 ± 0.1[a]	5.4 ± 0.4[b]
D	25.3 ± 0.5[a]	24.2 ± 0.0[b]	31.3 ± 1.2[c]
E	8.5 ± 0.2[a]	8.7 ± 0.2[a]	5.6 ± 0.2[b]
F	18.7 ± 0.2[a]	18.9 ± 0.1[a]	23.6 ± 1.1[b]
Total	1460.0 ± 330.8[a]	2763.3 ± 159.2[b]	2360.0 ± 248.5[b]
240°C			
A	15.7 ± 0.1	15.8 ± 0.1	15.5 ± 0.0
B	21.5 ± 0.1	21.5 ± 0.2	21.2 ± 0.1
C	7.4 ± 0.0	7.5 ± 0.1	7.4 ± 0.1
D	26.0 ± 0.1[a]	26.0 ± 0.1[a]	26.9 ± 0.4[b]
E	8.2 ± 0.1	8.4 ± 0.2	7.8 ± 0.3
F	21.2 ± 0.2	20.4 ± 0.2	21.3 ± 0.2
Total	18686.7 ± 737.3	18193.3 ± 1819.8	16240.0 ± 1430.0

[a]Structures of cyclic fatty acid monomers A–F are presented in Figure 1.
[b]Mixture of tripalmitin and trilinolenin (2:1) (PPP/LnLnLn, 2:1).
[c]Triacylglycerol (TG) made up of linolenic acid acylated in the *sn*-2 position and palmitic acid in the other two positions (PLnP).
[d]TG made up of linolenic acid acylated in one of the two outer *sn*-positions and palmitic acid in the other position (PPLn). Numbers with a different roman superscript in the same row are statistically different ($P < 0.05$).

TABLE 2
Content (ppm) of Cyclic Fatty Acid Monomers[a] Formed from Canola Oil and Its Interesterified Counterpart and Heated for 15 h at 180, 200, 220, and 240°C

	Original oil	Interesterified oil
180°C		
A	21.4 ± 1.4	24.3 ± 1.4
B	10.6 ± 0.3	9.0 ± 0.9
C	22.0 ± 1.9	24.4 ± 0.8
D	22.5 ± 0.5[b]	21.9 ± 0.8
E	1.8 ± 0.2	2.4 ± 0.3
F	21.5 ± 0.3[b]	18.1 ± 0.5
Total	9.0 ± 1.1	7.6 ± 0.5
200°C		
A	26.8 ± 1.4	39.2 ± 1.4
B	12.5 ± 0.4[b]	10.5 ± 0.1
C	21.5 ± 0.9[b]	16.0 ± 0.4
D	18.9 ± 1.1	15.0 ± 0.2
E	2.5 ± 0.2	2.4 ± 0.3
F	17.8 ± 0.3	16.8 ± 0.5
Total	40.0 ± 0.0[b]	30.0 ± 0.0
220°C		
A	19.1 ± 0.6	18.1 ± 0.2
B	21.6 ± 0.3	22.6 ± 0.2
C	9.2 ± 0.1	8.9 ± 0.0
D	23.3 ± 0.4[b]	23.0 ± 0.1
E	7.9 ± 0.4	8.1 ± 0.3
F	18.9 ± 0.2	19.2 ± 0.2
Total	465.0 ± 50.0[b]	346.0 ± 18.5
240°C		
A	17.6 ± 0.2	17.3 ± 0.2
B	22.1 ± 0.3[b]	21.9 ± 0.1
C	7.3 ± 0.3[b]	7.5 ± 0.2
D	24.8 ± 0.2[b]	25.0 ± 0.1
E	8.2 ± 0.0	8.2 ± 0.2
F	20.3 ± 0.1	19.9 ± 0.1
Total	2740.0 ± 113.0	2640.0 ± 297.0

[a]Structures of cyclic fatty acid monomers A–F are presented in Figure 1.
[b]$P < 0.05$.

ment, at least up to 220°C (Table 1). In addition, α-Ln in monoacid TG (LnLnLn) also exhibited a lower cyclization rate than in diacid TG (PLnP and PPLn) ($P < 0.05$, except at 240°C) (Table 1). Hence, in diacid TG containing 18:3n-3, the feature of *trans* geometrical isomerization was also observed for the CFAM formation (18), where PLnP is more sensitive to geometrical isomerization or CFAM formation than PPLn. Both geometrical isomerization (13) and cyclization (11) are thought to occur through a radical-mediated mechanism. Therefore, it is possible that the TG structure would influence the radical formation and thereby similarly affect both the cyclization and the geometrical isomerization rate. Nevertheless, whereas Ln in monoacid TG (LnLnLn) formed more *trans*-isomers than in diacid TG (PLnP and PPLn) (18), the reverse was observed for the CFAM formation (Table 1). Owing to the nonlinear spatial configuration of Ln, 18:3n-3 acylated in the three positions of the TG molecule would have greater steric hindrance for cyclization than when acylated in the diacid TG (PPLn and PLnP). Also, the formation of a CFAM in such a highly sterically hindered TG molecule would disturb the cyclization of the other Ln molecules. Therefore, based on these assumptions, the lower cyclization rate of α-Ln in LnLnLn should be due to steric hindrance, whereas in less-cluttered TG molecules such as PLnP and PPLn, the formation of CFAM would be more influenced by differences in radical stabilization. No other differences between the three model TG were observed at 240°C, probably because the energy (supplied by heating) necessary to bring about cyclization overwhelmed any other modulating factors.

TABLE 3
Content (ppm) of Cyclic Fatty Acid Monomers[a] Formed from Linoleic Acid in Monoacid and Diacid Triacylglycerols Heated for 30 h at 180, 200, 220, and 240°C

	PPP/LLL (2:1)[b]	PLP[c]	PPL[d]
180°C			
1	28.9 ±.2.7[a]	8.7 ± 0.7[b]	20.0 ± 1.7[c]
2	19.2 ± 1.3[a]	24.9 ± 0.8[b]	19.9 ± 0.4[a]
3	8.5 ± 0.9[a]	17.0 ± 1.8[b]	14.7 ± 1.1[b]
4	32.5 ± 1.4[a]	43.6 ± 2.7[b]	39.4 ± 0.5[b]
5	4.1 ± 0.7	3.6 ± 1.4	3.5 ± 1.0
6	8.0 ± 0.7[a]	2.3 ± 0.6[b]	2.5 ± 0.5[b]
Total	0.8 ± 0.2	1.9 ± 0.7	0.6 ± 0.2
200°C			
1	22.5 ± 1.3[a]	13.1 ± 1.4[b]	19.6 ± 1.3[a]
2	19.6 ± 0.8	20.3 ± 1.4	16.0 ± 1.3
3	13.6 ± 0.3[a]	19.8 ± 1.9[b]	16.3 ± 0.9[b]
4	35.2 ± 1.7	42.8 ± 1.8	43.1 ± 2.9
5	1.8 ± 0.6	1.8 ± 0.8	2.5 ± 0.9
6	7.3 ± 0.6[a]	2.3 ± 0.6[b]	2.6 ± 0.3[b]
Total	2.3 ± 0.1[a]	5.0 ± 1.2[b]	2.3 ± 0.2[a]
220°C			
1	19.5 ± 1.5	22.5 ± 0.4	19.5 ± 1.7
2	19.2 ± 1.8[a]	25.1 ± 1.2[b]	20.2 ± 0.8[a]
3	20.6 ± 0.6	18.2 ± 0.8	17.8 ± 1.5
4	35.8 ± 3.8	28.5 ± 1.5	33.7 ± 1.0
5	3.6 ± 0.1[a]	2.8 ± 0.5[a,b]	1.8 ± 0.2[b]
6	1.3 ± 0.1[a]	3.0 ± 0.5[a]	7.0 ± 1.9b
Total	45.6 ± 2.7[a]	45.1 ± 8.4[a,b]	24.5 ± 5.5[b]
240°C			
1	15.7 ± 1.1[a]	27.8 ± 1.4[b]	20.7 ± 0.2[b]
2	17.6 ± 1.9[a]	29.7 ± 1.5[b]	23.3 ± 0.3[c]
3	19.9 ± 1.1	18.0 ± 0.7	17.7 ± 0.6
4	39.9 ± 2.8[a]	18.6 ± 2.9[b]	25.5 ± 0.9[b]
5	5.0 ± 0.8	3.9 ± 1.0	6.5 ± 0.2
6	2.2 ± 0.2[a]	2.1 ± 0.1[a]	6.3 ± 0.6[b]
Total	349.9 ± 100.0	267.2 ± 48.0	346.7 ± 75.1

[a]Structures of cyclic fatty acid monomers 1–6 are presented in Figure 1.
[b]Mixture of tripalmitin and trilinolein (2:1) (PPP/LLL 2:1).
[c]Triacylglycerol made up of linoleic acid acylated in the *sn*-2 position and palmitic acid in the other two positions (PLP).
[d]Triacylglycerol made up of linoleic acid acylated in one of the two outer *sn*-positions and palmitic acid in the other positions (PPL). Numbers in the same row with a different roman superscript are statistically different ($P < 0.05$).

We then examined the CFAM products formed upon heating from a more realistic model consisting of canola oil and its randomized counterpart. These oils contained 18:3n-3 and 18:2n-6, both of which can form CFAM. The heating conditions applied were identical to those used for the model TG containing α-linolenate. Therefore, only the CFAM arising from 18:3n-3 can be accurately measured, as the cyclization rate of L is less than for Ln (Tables 1,3; Ref. 3).

As depicted in Table 2, the CFAM profiles of the original and the randomized oils were similar. On the other hand, at the two lowest temperatures (180 and 200°C) compound A accounted for much of the differences observed between the CFAM of the canola oils and of the model TG (Tables 1,3). Only compounds B and E are 18:3n-3 specific, whereas the other ones can be formed from both L and Ln (21). In addition, compound A may represent up to 55% of the CFAM formed from 18:2n-6 (21). Yet, as we mentioned earlier, a significant contribution of the CFAM formed from 18:2n-6 to the CFAM profile depicted in Table 2 is unlikely, especially at 180 and 200°C. Therefore, at these two temperatures, the differences in compound A content observed between the canola oils on the one hand and the model TG containing 18:3n-3 on the other hand cannot be ascribed to the presence of L in the canola oils. Presumably, an influence of the fatty acids co-acylated with Ln in canola oils (e.g., only P in the model TG system, and the combination between P, L, Ln, stearic and oleic acids in the canola oils system) would support this difference.

We previously determined that proportion of TG species containing more than one PUFA was greater in the original oil than in the interesterified one (19); the presence of 2–3 PUFA makes the TG molecule more sterically hindered in the former case than in the latter. Hence, based on the model TG study, less CFAM formation should occur in the original oil than in the interesterified counterpart. However, the results showed a departure from this hypothesis. In fact, in the original oil α-Ln was more concentrated in the *sn*-2 position (54%) than in the interesterified one (33%) (19). This result agrees with that found with the model TG, although the structural differences were less between the canola oils than between PLnP and PPLn. Therefore, in our experimental conditions, the TG structure seemed to be a more important factor than the slight differences in the TG composition to explain the differences in the CFAM formation between the two canola oils. Also, the influence of the structural factor was observed in the same range of temperatures between the model TG and the canola oils (e.g., up to 220°C). This structural factor may well account for the differences observed by Grandgirard and Julliard (10) between the CFAM formed from a canola oil and from a soybean oil heated for 10 to 45 h at 200°C. Both oils contained a similar amount of α-Ln (9.6 and 9.2%, respectively) but in the canola oil α-Ln is mainly acylated in the central position whereas it is randomly assigned in the soybean oil (27). According to our model, in the study of Grandgirard and Julliard (10) the canola oil was also more sensitive to CFAM formation (34 to 46%, depending on the heating time) than the soybean oil.

CFAM formed from α-Ln. Six peaks corresponding to CFAM were present in all the model TG samples and were quantified. Their identification was performed by GC–MS using DMOX derivatives, and their structures are illustrated in Figure 1B. A typical mass spectrum obtained from DMOX derivatives of the CFAM is featured in Figure 2. The size of the gap between two consecutive ions (between *m/z* 182 and 250), expressed in atomic mass unit (amu), is indicative of the ring size (28). The number of the ions separated by 14 amu and flanking the ring-gap region is indicative of the side-chain size (28).

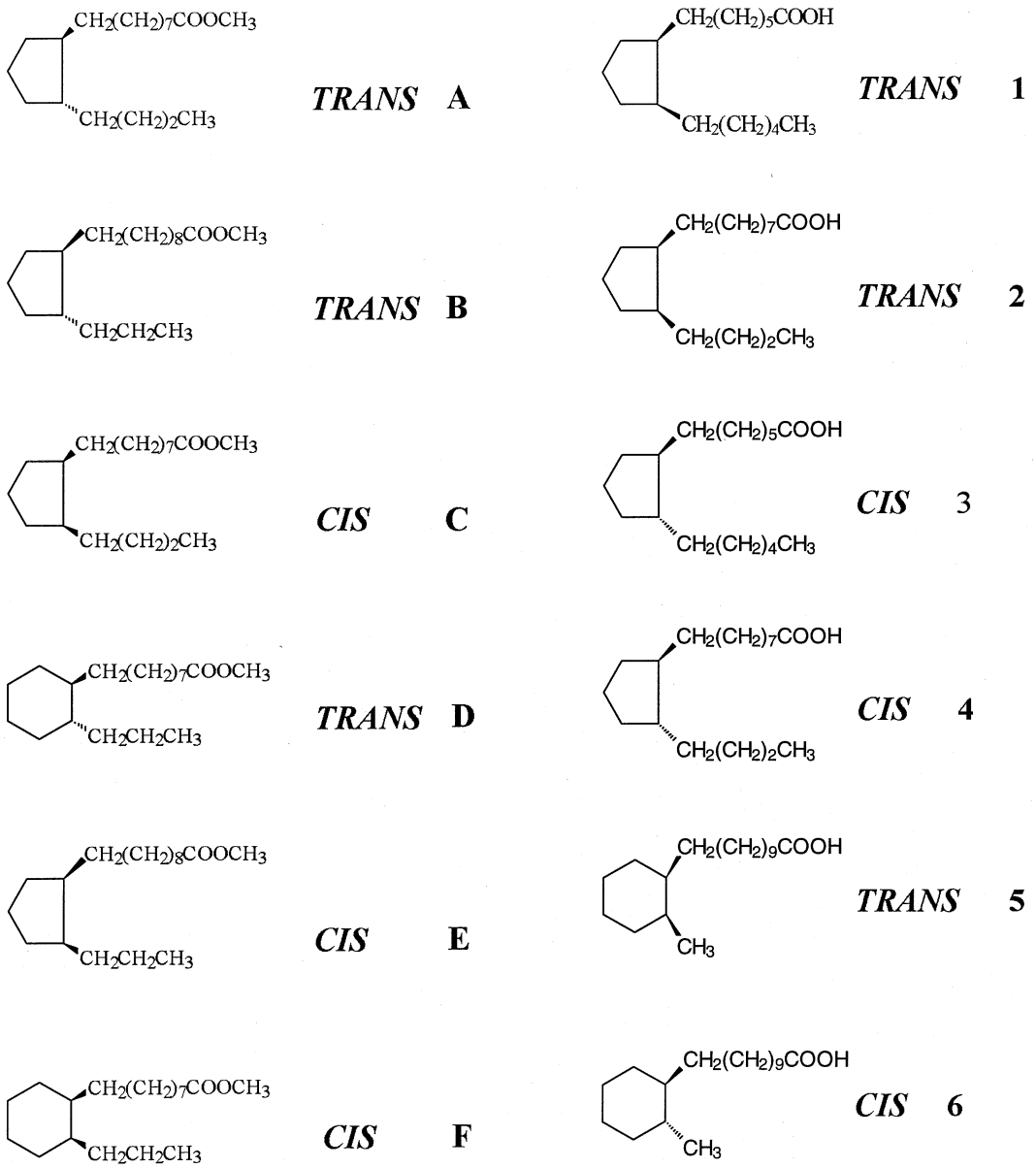

FIG. 1. Structures of cyclic fatty acid monomers (CFAM) determined after total hydrogenation. CFAM are presented according to their order of elution in gas chromatography as performed with a cyanopolysiloxane-type capillary column. CFAM denoted A–F are formed from 18:3n-3 and 1–6 are formed from 18:2n-6.

Compound 4 [methyl 9-(2′-butylcyclopentyl)-nonanoate] was the main CFAM detected in all model TG, except at 240°C for the diacid TG (PLP and PPL, Table 2).

Below 220°C, the amount of CFAM formed from L was very low (up to 5 ppm, Table 2). To ensure good accuracy in the procedure used for CFAM, quantitation required approximately 50 ppm of cyclic compound (21). Such content was obtained for all temperatures for the model TG containing α-Ln, for all temperatures but 180°C for the canola oils (Table 3), and only from 220°C for the model TG containing L (Table 2). Our experimental conditions did not allow us to obtain the formation of a larger amount of CFAM from L, as extended heating times (45 h, data not shown) did not improve CFAM formation. Others also observed such a limitation (10). Therefore, owing to the sensitivity of the technique, the observations done with L have to be considered more carefully than with α-Ln. However, comparison of the CFAM formation rate between the two diacid TG (PLP and PPL) indicates that L is twice as sensitive to heat-induced isomerization when it is acylated in the central position (PLP) than in one of the two outer positions (PPL). Also, there is a tendency for L in the monoacid TG (LLL) to form CFAM in amounts as low as PPL at the two lowest temperatures and as much as PLP at the two highest (Table 2).

No statistical differences were observed between the three model TG at 240°C, probably because the energy provided at this high temperature overwhelmed any other modulating factors involved in cyclization. It seems therefore that the position of L in the glycerol backbone is a more important factor

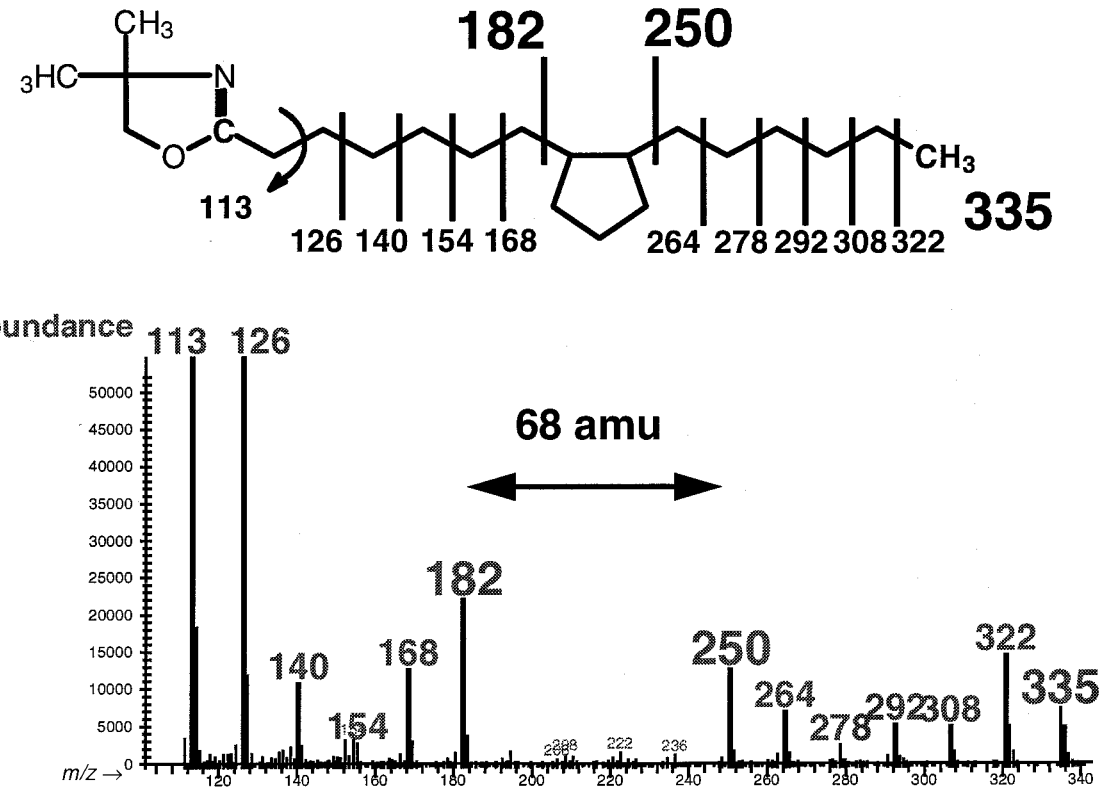

FIG. 2. Mass spectrum of a 4,4-dimethyloxazoline 7-(2′-hexylcyclopentyl)-heptanoate obtained after electron impact fragmentation.

governing CFAM formation than the number of L per TG molecule (see results at 220°C especially, Table 2). That is, L exhibits a greater resistance to heat-induced cyclization when it occupies one of the two outer positions in the TG molecule. We did not perform any experiments comparing the CFAM formation of an L-rich oil with its randomized counterpart, as with the canola oil in the present study. However, contrary to α-Ln, for which positional distribution may vary according to the plant species, L is predominantly found in the central position of TG in plants. In this location, one may assume that, unless genetically, enzymatically or chemically modified, the natural positioning of the linoleoyl moiety in the central position of TG would favor its cyclization upon heat treatment.

In conclusion, both the TG composition and the TG structure were found to influence thermal formation of lipid byproducts, but in a direction that may vary between the oligomer (19), the *trans*-isomers (18), and the CFAM (present study). Hence, besides the fatty acid composition, the TG factor should be taken into account to determine the sensitivity of a given oil to heat treatment.

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