Effect of Fatty Acid Positional Distribution and Triacylglycerol Composition on Lipid By-Products Formation During Heat Treatment: II. *Trans* Isomers

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ABSTRACT: This study examined the effect of the fatty acid positional distribution and of the triacylglycerol (TG) composition on heat-induced *trans* isomerization of linoleic and linolenic acids. For this, we synthesized diacid TG molecules that were acylated only with linoleic acid (L) or with linolenic acid (Ln) along with palmitic acid (P). The fatty acid of interest was positioned either in the central position (PLP and PLnP, respectively) or in one of the two outer positions (PPL and PPLn, respectively). Monoacid TG, i.e., trilinolein and trilinolenin, were also synthesized and mixed with tripalmitin in a 1:2 ratio. This model TG was also compared to another TG model, which 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. After heating, the content of *trans* isomers was determined by gas–liquid chromatography with a polar capillary column. In model TG, polyunsaturated fatty acids in monoacid TG (LLL and LnLnLn) exhibited the highest degree of isomerization, compared to diacid TG, and this effect was greatest at 220°C. At this temperature, an effect of the TG structure was observed only with linolenic acid. In that situation, 18:3n-3 acylated in the central position of the TG molecule (PLnP) displayed the highest sensitivity to *trans* geometrical isomerization. Although to a lesser extent, the same trends as for the pure TG model were observed with the canola oil model with regard to the influence of the fatty acid positional distribution and TG molecular species.
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KEY WORDS: Canola oil, heat treatment, interesterified oil, TG composition, TG structure, *trans* polyunsaturated fatty acids.

Trans geometrical isomers of essential fatty acids of 18-carbon chainlength (linoleic acid, 18:2n-6, and linolenic acid, 18:3n-3) were identified as early as 1974 (1). Since that time, they have been detected in several foodstuffs, from vegetable oils and low-calorie spreads (2) to infant formulas (3,4). They originate from the heat treatment carried out during deep-fat frying and industrial refining of oils by a thermally induced mechanism (5). Their presence in food is of concern because they have been shown to be desaturated and chain-elongated into higher metabolites (6–9). These latter can modify physiological functions in comparison to their *cis* homologues, such as platelet aggregation and eicosanoid metabolism (10,11). They also caused changes in the electroretinograms of rat pups whose mothers were fed *trans* n-3 fatty acids (12). Given their occurrence and their nutritional implications (12,13), it is important to determine factors that may influence the formation of these *trans* polyunsaturated fatty acids (PUFA). The main factors identified so far are temperature (5,14–16), heating time (5,14–16), and degree of unsaturation (16). Nevertheless, other parameters seem to affect the isomerization rate of both 18:2n-6 and 18:3n-3. Likewise, O'-Keefe *et al.* (17) reported a higher degree of isomerization (DI) for both 18:2n-6 and 18:3n-3 in marketed canola oils than in soybean oils. The same kind of differences for these oils was observed by others after heating under laboratory conditions (16), although some studies did not report such a finding (14,15).

Beside the unsaponifiable compounds, oil compositions differ not only by the nature and the proportion of their constituent fatty acids but also by their arrangement on the triacylglycerol molecules (TG). Both TG structure and TG composition can affect the physicochemical properties of oils. The oxidative stability of synthetic (18,19) and purified canola oil TG (20) or soybean oil TG (21) have been shown to be affected by these two factors. The oxidation of PUFA occurs *via* a radical mechanism (22). Wolff (23) hypothesized that heat-induced isomerization of methylene-interrupted double bonds takes place *via* a radical mechanism. Therefore, it is conceivable that the different sensitivity of PUFA to heat-induced geometrical isomerization in natural oils could be influenced by differences in their TG structure and TG molecular species.

The present study was intended to clarify this issue. For this, we determined the DI of both linoleic and linolenic acids when esterified in model TG or esterified in TG of a canola oil and its interesterified counterpart.

EXPERIMENTAL PROCEDURES

Pure model TG and canola oil TG. 1,2-Dipalmitoyl-3-linolein (PPL), 1,2-dipalmitoyl-3-linolenin (PPLn), 1,3-dipalmitoyl-2-

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linolein (PLP), 1,3-dipalmitoyl-2-linolenin (PLnP), and mixtures of trilinolein (LLL) and tripalmitin (PPP) (1:2) and of trilinolenin (LnLnLn) and PPP (1:2) were synthesized as described elsewhere (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 part I of this series of reports (24). They were composed of $C_{16:0}$ (8.0%), $C_{18:0}$ (4.9%), $C_{18:1}\Delta 9c$ (59.0%), $C_{18:1}\Delta 11c$ (2.8%), $C_{18:2}\Delta 9c,12c$ (18.7%), and $C_{18:3}\Delta 9c,12c,15c$ (6.3%).

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 linolenic acid) or 30 h (canola oils and model TG with linoleic acid). Each TG sample was heated 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.

Structural analysis and TG composition analysis. The method used to carry out both stereospecific and TG molecular species analyses of the canola oils has been detailed by Martin *et al.* (24). It is based on reversed-phase HPLC and chiral-phase HPLC, respectively.

Preparation of fatty acid methyl esters (FAME). An aliquot (5 mg) of each of the heated TG was transesterified with boron trifluoride in methanol (14%, vol/vol) as described before (26). The resulting FAME were extracted with 2 × 2 mL hexane and 2 mL water and dried over Na_2SO_4. The samples in solvent were then stored at −20°C prior to gas–liquid chromatography (GLC) analysis.

Gas chromatographic analysis. FAME analysis 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) in programming mode. 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 for 5 min. Quantitation was performed by peak integration with a Spectra-Physics Chromjet integrator and a Spectra-Physics Chemstation that was operated by WOW software (Spectra-Physics, La Verpillière, France). *Trans* isomers were identified from the 18:2Δ9*c*,12*c* isomers of a sunflower oil and from the 18:3Δ9*c*,12*c*,15*c* isomers of a heated linseed oil, as detailed in analyses and isomer determinations already performed in our laboratory (27,28).

Statistics. Results were computed with Microsoft Excel and statistically evaluated with SigmaStat® (Jandel Scientific, San Rafael, CA). Results were expressed as means ± SEM (n = 3 for each sample). For multiple comparisons (pure model TG groups), significance of the differences between the means found for the isomers was analyzed by analysis of variance on ranks. Student's 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

Our study describes the influence of TG structure and TG composition on the formation of *trans* isomers of linoleic and linolenic acids upon heat treatment. We chose temperatures that are representative of common frying operations (180 and 200°C) and of industrial refining conditions (220 and 240°C).

Figure 1 depicts partial chromatograms of methyl esters of pure TG of 18:2Δ9*c*,12*c* (panel A) and of 18:3Δ9*c*,12*c*,15*c* (panel B) heated at 240°C for 30 h and 15 h, respectively. The 18:2Δ9*c*,12*c* isomers identified were 18:2Δ9*t*,12*t*, 18:2Δ9*c*,12*t* and 18:2Δ9*t*,12*c*, and those of 18:3Δ9*c*,12*c*,15*c* were 18:3Δ9*t*,12*t*,15*t*, 18:3Δ9*c*,12*t*,15*t*, 18:3Δ9*t*,12*c*,15*t*, unresolved 18:3Δ9*c*,12*c*,15*t* and 18:3Δ9*t*,12*t*,15*c*, 18:3Δ9*c*,12*t*,15*c*, and 18:3Δ9*t*,12*c*,15*c*.

Pure model TG study. Table 1 outlines the isomer composition of linoleic acid, acylated in three different types of TG molecules and determined for different temperatures. The *trans* isomers were formed at temperatures as low as 180°C,

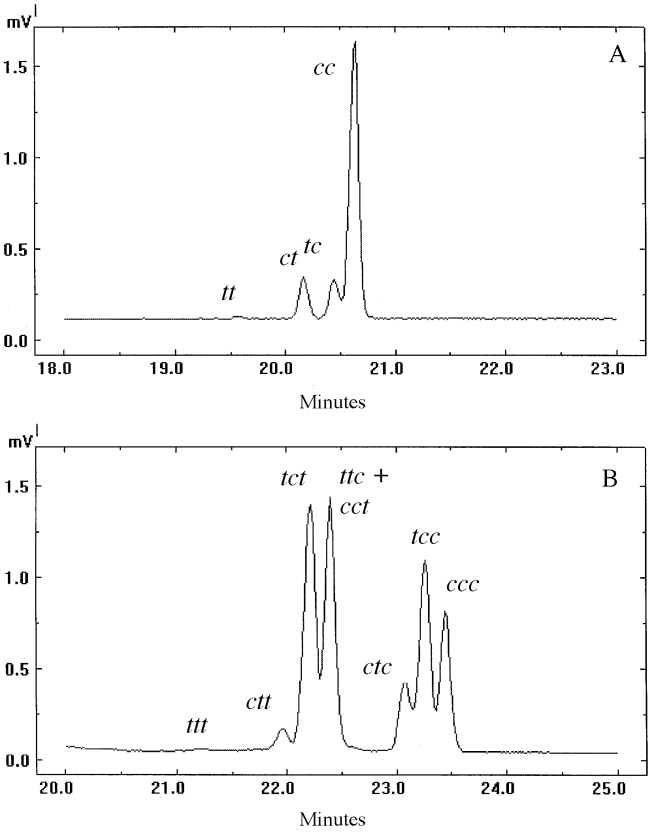

FIG. 1. Partial chromatogram of 18:2Δ9*c*,12*c* (A) and 18:3Δ9*c*,12*c*,15*c* (B) heated at 240°C for 30 h and 15 h, respectively. (A) *tt*, 18:2Δ9t,12*t*; *ct*, 18:2Δ9*c*,12*t*; *tc*, 18:2Δ9*t*,12*c*; *cc*, 18:2Δ9*c*,12*c*. (B) *ttt*, 18:3Δ9*t*,12*t*,15*t*; *ctt*, 18:3Δ9*c*,12*t*,15*t*; *tct*, 18:3Δ9*t*,12*c*,15*t*; *ttc* + *cct*, 18:3Δ9t,12*t*,15*c* + 18:3Δ9*c*,12*c*,15*t*; *ctc*, 18:3Δ9*c*,12*t*,15*c*; *tcc*, 18:3Δ9*t*,12*c*,15*c*; *ccc*, 18:3Δ9*c*,12*c*,15*c*.

TABLE 1
Relative Content of *trans* Geometrical Isomers of Linoleic Acid in Monoacid and Diacid Triacylglycerols (TG) Heated for 30 h at 180, 200, 220, and 240°C[a]

	PPP/LLL	PLP	PPL
180°C			
tt	0.15 ± 0.01	n.d.	n.d.
ct	0.18 ± 0.03	0.17 ± 0.01	0.19 ± 0.02
tc	0.20[a] ± 0.01	0.12[b] ± 0.01	0.10[b] ± 0.01
cc	99.50[a] ± 0.05	99.71[b] ± 0.01	99.70[b] ± 0.01
Total isomers	0.53[a] ± 0.05	0.29[b] ± 0.01	0.30[b] ± 0.01
200°C			
tt	0.15 ± 0.02	n.d.	n.d.
ct	0.30 ± 0.01	0.28 ± 0.02	0.27 ± 0.02
tc	0.38[a] ± 0.04	0.21[b] ± 0.01	0.19[b] ± 0.03
cc	99.17[a] ± 0.02	99.51[b] ± 0.01	99.54[b] ± 0.04
Total isomers	0.82[a] ± 0.02	0.49[b] ± 0.01	0.46[b] ± 0.04
220°C			
tt	0.45[a] ± 0.04	0.23[b] ± 0.01	0.19[b] ± 0.02
ct	3.80[a] ± 0.17	2.90[b] ± 0.02	3.06[b] ± 0.15
tc	3.64[a] ± 0.14	2.69[b] ± 0.07	2.27[b] ± 0.50
cc	92.11[a] ± 0.34	94.15[b] ± 0.11	94.48[b] ± 0.67
Total isomers	7.81[a] ± 0.34	5.85[b] ± 0.11	5.52[b] ± 0.67
240°C			
tt	1.42[a] ± 0.28	0.38[b] ± 0.11	0.63[b] ± 0.02
ct	11.85 ± 0.18	11.46 ± 0.23	11.60 ± 0.21
tc	11.61 ± 0.45	11.16 ± 0.22	11.56 ± 0.25
cc	75.12 ± 0.89	76.98 ± 0.52	76.21 ± 0.47
Total isomers	24.88 ± 0.89	23.02 ± 0.52	23.79 ± 0.47

[a]Mixture of tripalmitin and trilinolein (2:1) (PPP/LLL 2:1); TG made up of linoleic acid acylated in the *sn*-2 position and palmitic acid in the two other positions (PLP); and TG made up of linoleic acid acylated in one of the two outer *sn* positions and palmitic acid in the other positions (PPL). Numbers with different superscript letters in the same row are statistically different ($P < 0.05$); n.d., not detected.

TABLE 2
Relative Content of *trans* Geometrical Isomers of Linolenic Acid in Monoacid and Diacid TG Heated for 15 h at 180, 200, 220, and 240°C[a]

	PPP/LnLnLn	PLnP	PPLn
180°C			
ttt	n.d.	n.d.	n.d.
ctt	n.d.	n.d.	n.d.
tct	n.d.	n.d.	n.d.
cct + ttc	0.42 ± 0.16	0.61 ± 0.20	0.48 ± 0.06
ctc	n.d.	n.d.	n.d.
tcc	n.d.	n.d.	n.d.
ccc	99.55 ± 0.19	99.28 ± 0.27	99.42 ± 0.06
Total isomers	0.42 ± 0.16	0.61 ± 0.20	0.48 ± 0.06
200°C			
ttt	n.d.	n.d.	n.d.
ctt	n.d.	n.d.	n.d.
tct	n.d.	n.d.	0.16 ± 0.08
cct + ttc	2.13 ± 0.16	2.54 ± 0.23	2.02 ± 0.25
ctc	0.17 ± 0.09	0.52 ± 0.02	0.52 ± 0.17
tcc	1.65[a] ± 0.18	2.23[a] ± 0.27	0.82[b] ± 0.13
ccc	96.06 ± 0.24	94.64 ± 0.25	96.48 ± 0.60
Total isomers	3.94 ± 0.24	5.03 ± 0.25	3.52 ± 0.60
220°C			
ttt	0.59[a] ± 0.32	n.d.	0.10[b] ± 0.02
ctt	1.41[a] ± 0.58	0.72[b] ± 0.31	0.35[b] ± 0.03
tct	10.37[a] ± 0.95	6.09[b] ± 0.40	3.97[c] ± 0.06
cct + ttc	25.75[a] ± 0.54	22.43[b] ± 0.60	19.32[c] ± 0.10
ctc	5.84[a] ± 0.66	3.87[b] ± 0.33	2.69[b] ± 0.05
tcc	20.05[a] ± 0.93	18.34[a] ± 0.40	16.19[b] ± 0.60
ccc	36.00[a]± 2.14	48.56[b] ± 1.66	57.49[c] ± 0.53
Total isomers	64.00[a] ± 2.14	51.44[b] ± 1.66	42.51[c] ± 0.53
240°C			
ttt	0.33 ± 0.06	0.15 ± 0.08	0.19 ± 0.01
ctt	2.69 ± 0.37	2.84 ± 0.28	2.78 ± 0.50
tct	30.52 ± 0.73	31.53 ± 1.57	29.13 ± 0.06
cct + ttc	25.73 ± 0.29	25.43 ± 1.01	26.20 ± 0.49
ctc	7.99 ± 0.37	8.22 ± 0.45	7.56 ± 0.20
tcc	20.39 ± 0.76	20.09 ± 0.35	20.25 ± 0.67
ccc	12.35 ± 1.03	11.90 ± 0.62	13.91 ± 0.45
Total isomers	87.65 ± 1.03	88.08 ± 0.65	86.10 ± 0.45

[a]Mixture of tripalmitin and trilinolenin (2:1) (PPP/LLL 2:1), TG made up of linolenic acid acylated in the *sn*-2 position and palmitic acid in the two other positions (PLnP); and TG made up of linolenic acid acylated in one of the two outer *sn* positions and palmitic acid in the other positions (PPLn). Numbers with different superscript letters in the same row are statistically different ($P < 0.05$). See Table 1 for abbreviations.

and their contents increased with temperature as already reported (5,16). This increase was not steady, and the temperature of extensive formation of *trans* isomers starts at 220°C. Aside from this overall sensitivity of 18:2Δ9*c*,12*c* for isomerization upon heating, differences were observed between isomeric TG. For instance, the DI was always higher for the monoacid TG (LLL) than for the diacid TG (PPL and PLP) ($P < 0.05$), except at 240°C. Moreover, for the mono-*trans* isomers, the *trans*-isomerization occurred in a higher proportion at carbon-9 (18:2Δ9*t*,12*c*) for the monoacid TG (LLL) than for the two diacid TG (PPL and PLP) ($P < 0.05$ at 180, 200, and 220°C). Finally, the level of di-*trans* isomer (18:2Δ9*t*,12*t*) was also higher in the monoacid than in diacid TG ($P < 0.05$). Altogether, this indicates that linoleic acid is more sensitive to *cis*/*trans* isomerization when it is acylated in monoacid TG than in diacid TG, whatever its positional distribution, and that this is mainly due to a higher rate of isomerization of the double bond located at carbon 9 of linoleic acid.

Results for linolenic acid (18:3Δ9*c*,12*c*,15*c*) are presented in Table 2. As already reported by others (5), the DI of 18:3Δ9*c*,12*c*,15*c* increased with temperature, and 220°C was the determining point for increased formation of *trans* isomers. This temperature merely allowed us to observe statistically significant differences between model TG. At this temperature, as observed with linoleic acid, linolenic acid esterified in monoacid TG (LnLnLn) was much more sensitive to isomerization (64%) than in diacid TG (PPLn and PLnP) (Table 2). However, in contrast to 18:2Δ9*c*,12*c*, significant differences were also observed between the two diacid TG. Linolenic acid acylated in the central position of monoacid TG (PLnP) was also more sensitive to isomerization than when acylated in one of the two outer positions (PPLn) ($P < 0.05$ at 220°C). It is not known if this holds true in the monoacid TG (LnLnLn). The DI at 220°C was therefore in

the decreasing order LnLnLn > PLnP > PPLn, and this applied also to each *trans* isomer we detected in a sufficient amount to be accurately measured (Table 2).

Wolff *et al.* (23) postulated a delocalization of the electrons of the double bond after the formation of an unstable radical. It is thus conceivable that both the nature and the relative location of the substituting fatty acids near the PUFA of 18-carbon chainlength would exert a different stabilizing ability on the radical, through mesomeric effects for instance, and thereby influence the isomerization rate. This is also supported by the different sensitivity for isomerization of the double bond located at carbon-9 of linoleic acid in monoacid and diacid TG. This double bond is the closest to the carbonyl linked to glycerol and also theoretically the most sensitive to the influence of mesomeric or inductive effects that originate from other substituents in the glycerol backbone. Wolff *et al.* (5) noticed differences in the rate of isomerization of linolenic acid also when heated in the TG (87%) or methyl ester form (78%). This again emphasizes the importance of the substituent linked to the carbonyl end of the PUFA to modulate isomer formation.

The effect of both TG composition and structure was mainly observed at 220°C for 18:3Δ9*c*,12*c*,15*c*, whereas significant differences were detected at 180, 200, and 220°C for 18:2Δ9*c*,12*c*. This might be paradoxical because linolenic acid is 13 to 14 times more sensitive to isomerization than linoleic acid (17,29). However, in our study, the heating time was shorter for 18:3Δ9*c*,12*c*,15*c* (15 h) than for 18:2Δ9*c*,12*c* (30 h). In spite of this, the DI was always higher for linolenic acid than for linoleic acid (Tables 1 and 2), as reported by others (17,30).

Canola oil study. In the original canola oil, both 18:2Δ9*c*,12*c* and 18:3Δ9*c*,12*c*,15*c* were acylated mainly in the *sn*-2 position, to a lesser extent in the *sn*-1, and least in the *sn*-3 position (see Table 5 in Ref. 24). Conversely, all fatty acids were randomly assigned to each *sn*-position in the interesterified oil [33% in each position (24)]. Table 3 reports the profiles of the 18:2Δ9*c*,12*c* isomers in the original and in the randomized canola oil. For both oils, the isomer profile was dependent on the temperature. At the two lowest temperatures (180 and 200°C), the DI of both canola oils was intermediary between the two extreme values found with the model TG. On the other hand, the DI was lower at the two highest temperatures (Tables 1 and 3). This again emphasizes the finding substantiated with the model TG, which demonstrates that, for 18:2Δ9*c*,12*c*, the nature of the fatty acid coesterified with 18:2Δ9*c*,12*c* in the TG molecule (linoleate or palmitate for the model TG, palmitate and the other fatty acids depicted in Table 6, Ref. 24 for the canola oils) would influence the isomerization rate of this fatty acid. Nevertheless, no differences were observed between the original and the interesterified canola oils (Table 3), thereby indicating that large differences in TG fatty acid composition are required to affect significantly the isomerization rate of linoleic acid.

As observed with linoleic acid, linolenic acid in the canola oils (Table 4) generally had a lower DI than in the model oil

TABLE 3
Relative Content of *trans* Geometrical Isomers of Linoleic Acid in the Original and in the Interesterified Canola Oils TG Heated for 30 h at 180, 200, 220, and 240°C

	Original oil[a]	Interesterified oil
180°C		
tt	n.d.	n.d.
ct	0.42 ± 0.02	0.41 ± 0.02
tc	n.d.	n.d.
cc	99.58 ± 0.02	99.59 ± 0.02
Total isomers	0.42 ± 0.02	0.41 ± 0.02
200°C		
tt	n.d.	n.d.
ct	0.34 ± 0.04	0.44 ± 0.12
tc	0.30 ± 0.04	0.28 ± 0.02
cc	99.36 ± 0.07	99.29 ± 0.13
Total isomers	0.64 ± 0.07	0.71 ± 0.13
220°C		
tt	n.d.	n.d.
ct	1.60 ± 0.16	1.57 ± 0.03
tc	1.47 ± 0.12	1.59 ± 0.11
cc	96.93 ± 0.26	96.83 ± 0.13
Total isomers	3.07 ± 0.26	3.17 ± 0.13
240°C		
tt	0.35 ± 0.02	0.30 ± 0.07
ct	6.97 ± 0.18	6.91 ± 0.25
tc	6.66 ± 0.16	6.77 ± 0.27
cc	86.02 ± 0.36	86.02 ± 0.59
Total isomers	13.98 ± 0.36	13.98 ± 0.59

[a]$P > 0.05$ between the two oils. See Table 1 for abbreviations.

TG (Table 2), although the differences were less than observed with 18:2Δ9*c*,12*c*. By analogy, similar conclusions on the isomerization rate of 18:3Δ9*c*,12*c*,15*c* might be drawn for the influence of the fatty acids co-esterified with linolenic acid in the single TG molecules. Additionally, compared to the randomized oil, linolenic acid is mainly acylated in the central position in the original oil (33 vs. 55%, respectively) (24). As observed with the model TG (Table 2), this positioning of 18:3Δ9*c*,12*c*,15*c* would explain its higher DI in the original oil than in the randomized one (Table 4). Also, the dilinolenoyl TG species was higher in the original than in the randomized product (1.1 vs. 0.6%) (24). Then, based on the model TG experiment, one cannot rule out the possibility that the differences in the TG species between the randomized and the original canola oils can also explain part of the differences observed in their *trans* linolenic acid content.

The PUFA positional distribution within TG could be the main reason for the higher DI 18:3Δ9*c*,12*c*,15*c*/DI 18:2Δ9*c*,12*c* ratios in commercial canola oils than in commercial soybean oils found by O'Keefe *et al.* (17); 18:3Δ9*c*,12*c*,15*c* is mainly present in the central position in the canola oil TG (24), whereas it is randomly assigned to each of the three *sn*-positions of TG in soybean oil (31). Grandgirard and Julliard (16) also found that the DI of 18:3Δ9*c*,12*c*,15*c* was slightly higher in a canola oil heated at 240°C for 10 h (77.3%) than in a soybean oil heated under the

TABLE 4
Relative Content of *trans* Geometrical Isomers of Linolenic Acid in the Original and in the Interesterified Canola Oils TG Heated for 15 h at 180, 200, 220, and 240°C

	Original oil	Randomized oil
180°C		
ttt	n.d.	n.d.
ctt	n.d.	n.d.
tct	n.d.	n.d.
cct + ttc	1.02 ± 0.01	1.27 ± 0.13
ctc	n.d.	n.d.
tcc	n.d.	n.d.
ccc	98.98 ± 0.01	98.73 ± 0.13
Total isomers	1.02 ± 0.01	1.27 ± 0.13
200°C		
ttt	n.d.	n.d.
ctt	n.d.	n.d.
tct	n.d.	n.d.
cct + ttc	2.44[a] ± 0.10	2.18 ± 0.07
ctc	n.d.	n.d.
tcc	2.44 ± 0.10	2.18 ± 0.07
ccc	96.76[a] ± 0.08	97.13 ± 0.08
Total isomers	3.24[a] ± 0.08	2.87 ± 0.08
220°C		
ttt	n.d.	n.d.
ctt	n.d.	n.d.
tct	4.22[a] ± 0.13	3.57 ± 0.11
cct + ttc	21.13[a] ± 0.20	19.77 ± 0.27
ctc	2.62[a] ± 0.07	2.24 ± 0.05
tcc	16.76[a] ± 0.10	15.82 ± 0.16
ccc	55.27[a] ± 0.39	58.60 ± 0.55
Total isomers	44.73[a] ± 0.53	41.40 ± 0.55
240°C		
ttt	n.d.	n.d.
ctt	1.82 ± 0.26	1.40 ± 0.10
tct	22.23[a] ± 0.49	19.84 ± 0.18
cct + ttc	28.32 ± 0.12	28.13 ± 0.24
ctc	6.94[a] ± 0.22	6.10 ± 0.18
tcc	21.49 ± 0.29	22.22 ± 0.17
ccc	19.20[a] ± 0.55	22.31 ± 0.21
Total isomers	80.80[a] ± 0.55	77.69 ± 0.21

[a]$P < 0.05$. See Table 1 for abbreviations.

same conditions (74.4%). Hence, based on our conclusion, the preferred location of 18:3Δ9*c*,12*c*,15*c* in the *sn*-2 position of natural canola oil TG would make it more susceptible to isomerization than in soybean oil TG, where it is more randomly assigned to each *sn*-position. However, although the highest values of *trans* isomers were found in marketed canola oils both in the United States and in Europe, as recently reviewed by O'Keefe (17), contradictory results (14) or no differences (15) were also found in studies carried out under conditions of controlled temperatures. From that and from our present results, one may gather that, for natural oils, TG composition and structure exert only a slight effect on the formation of isomeric 18:2Δ9*c*,12*c* and 18:3Δ9*c*,12*c*,15*c* products. Nevertheless, it appears from the present data that the main influence of these factors is seen at 220°C, a temperature frequently used during industrial deodorization. In this situation, the pure model TG experiment indicates that the PUFA in monoacid TG were more susceptible to isomerization than in diacid TG. In diacid TG, only linolenic acid presented differences in the isomerization rate according to its positional distribution. Then, the *sn*-2 position was more sensitive to heat-induced isomerization than the two outer positions. The same trends as for the pure model TG were observed with the canola oil models, but to a lesser extent.

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