Evolution of Short-Chain Glycerol-Bound Compounds During Thermoxidation of FAME and Monoacid TAG

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ABSTRACT: Methyl heptanoate and octanoate, methyl 8-oxo-octanoate and 9-oxononanoate, and dimethyl suberate and azelate were quantitated in model systems of FAME, i.e., methyl oleate and linoleate, and monoacid TAG, i.e., triolein and trilinolein, heated at 180°C for 5, 10, and 15 h. Polar compounds, ranging from 20.2 to 53.2% in TAG systems, were also quantitated as a reference for the level of degradation of the samples analyzed. Base-catalyzed techniques were used for methylation as they did not modify the structure of the compounds of interest. The maximum amounts of short-chain FAME and of aldehydic FAME obtained were of the same order (3–4 mg/g sample in methyl linoleate and trilinolein heated for 15 h). After a second methylation step with diazomethane, amounts of dimethyl suberate and azelate were also quantitated. The total amounts of dimethyl esters in TAG model systems ranged from 1.07 to 3.35 mg/g of heated sample and were similar to those found for total aldehydic FAME (from 1.03 to 3.15 mg/g of heated sample), suggesting that oxidation of the aldehydic acyl group to an acid acyl group was a major reaction.

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KEY WORDS: Aldehydic acids, diacids, fatty acids, methylation, short-chain glycerol-bound compounds, thermoxidation.

During deep fat frying, hydroperoxides decompose into a wide range of volatile and nonvolatile secondary oxidation products (1–4). From a nutritional point of view, the nonvolatile degradation products present in used frying fats are of importance because they remain in the oil, subsequently being absorbed into the food and thereby ingested. Among them, oxidized TAG monomers are of particular interest because they have a high degree of digestibility (5–7).

An important route of hydroperoxide breakdown proceeds by homolytic β-scission of the alkoxy radicals coming from allylic hydroperoxides to form ultimately volatile and nonvolatile oxidation compounds, the latter including short-chain glycerol-bound aldehydes (2). Information available so far regarding the analysis of these ester-bound aldehydes, also called core aldehydes, is limited to qualitative data. The reports on this subject published during the last few years focused on methods for detection of oxidation products in intact TAG, phospholipids, and cholesteryl esters, which have been very useful to demonstrate the presence of aldehydes in lipoproteins and in biomembranes prepared from tissues (8–12). However, these papers provide no quantitative data on the level of core aldehydes.

Core aldehydes are absorbed after being hydrolyzed to short-chain aldehydic acids by the action of pancreatic lipase. Some reports on 9-oxononanoic acid, the major aldehydic acid in oxidized lipids, indicate that it could induce hepatic lipid peroxidation (13) and affect hepatic metabolism (14,15). Consequently, quantitation of the total amount of aldehydic acids in dietary lipids is more relevant than the analysis of intact molecules.

In a previous paper, we reported qualitative analysis of aldehydic acids as FAME derivatives in frying oils by GLC–MS (16), and the need arose for deeper studies on derivatization methods for quantitative purposes. Recently, we studied the different methylation procedures and selected two base-catalyzed methods as the most suitable for GLC quantitation of major short-chain glycerol-bound compounds, particularly ester-bound aldehydes (17).

The objective of the present work was to study the evolution of major short-chain glycerol-bound compounds during thermoxidation of model systems of FAME and TAG constituted by a single FA. Methyl linoleate and oleate as well as trilinolein and triolein were selected as model compounds because linoleic and oleic acids are the most representative FA undergoing changes during deep fat frying. Model systems of FAME were used as the reference model system since they can be analyzed by GLC and GLC–MS without further derivatization.

EXPERIMENTAL PROCEDURES

Samples and reagents. Methyl linoleate (ML, 18:2n-6), methyl oleate (MO, 18:1n-9), trilinolein (LLL), triolein (OOO), methyl octanoate (C8:0), methyl tridecanoate (C13:0), and methyl pentadecanoate (C15:0) were purchased from Nu-Chek-Prep (Elysian, MN). Silica gel 60 for column chromatography (particle size 0.063–0.100 mm) was purchased from Merck (Darmstadt, Germany). Azelaic acid (nonanodioic acid) and suberic acid (octanodioic acid) were obtained from Sigma (St. Louis, MO). All other solvents and chemicals (of reagent grade or better quality) were obtained from local suppliers.

Heating procedure. MO, ML, OOO, or LLL (2 ± 0.01 g) were weighed directly into standard glass tubes (20 cm × 12 mm i.d.). Tubes were introduced into a Rancimat vessel containing 8 g of glycerol to facilitate heat transfer, and inserted into the heating block previously heated to 180 ± 1°C. Vessels were

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left open during heating. Samples were heated for 5, 10, and 15 h in duplicate and kept at –20°C until analyses. This procedure was described in detail, including data repeatability, in a recent publication (18).

Analytical methods. (i) Quantitation of total polar compounds and their distribution. Nonpolar and polar fractions were separated from 1 g of sample by silica column chromatography (20 g silica H_2O, 95:5, w/w). Nonpolar fractions containing unoxidized TAG or FAME were eluted with 150 mL of *n*-hexane/diethyl ether (90:10, vol/vol) for OOO and LLL (19) and 150 mL of *n*-hexane/diethyl ether (95:5, vol/vol) for ML and MO (20). A second fraction, comprised of total polar compounds, was eluted with 150 mL of diethyl ether. Efficiency of the separation by adsorption chromatography was checked by TLC using hexane/diethyl ether/acetic acid (80:20:1, by vol) for development of plates and exposure to iodine vapor to reveal the spots. After evaporation of solvents, both fractions were weighed and dissolved in di-isopropyl ether until further analysis. The polar and nonpolar fractions obtained were dissolved in di-isopropyl ether (25 mg/mL) for analysis by high-performance size exclusion chromatography (HPSEC), using a Rheodyne 7725i injector with 10-μL sample loop, a Waters 510 pump (Waters, Milford, MA), an HP 1037 A refractive index detector, and an HP 3392 A integrator (Hewlett-Packard, Avondale, PA). The separation was performed on two 100 and 500Å Ultrastyragel columns (25 cm × 0.77 cm i.d.) packed with porous, highly cross-linked styrene–divinylbenzene copolymers (film thickness 10 μm) (Hewlett-Packard) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase (19).

(ii) Quantitation of short-chain glycerol-bound compounds. A standard solution was prepared with a mixture of C13:0 (500 μg/mL) and C15:0 (500 μg/mL) FAME in diethyl ether or *tert*-butylmethyl ether (TBME). The following methylation procedures were applied.

Base-catalyzed transmethylation with 2 N potassium hydroxide in methanol at room temperature: 100 mg of heated FAME or TAG was accurately weighed into a screw-capped centrifuge tube and dissolved in 0.5 mL of the standard solution in diethyl ether. Potassium hydroxide in methanol (0.1 mL, 2N) was then added, and the vial was closed and shaken vigorously for 30 s. One milliliter of brine solution was added and the solution was neutralized with acetic acid. The mixture was shaken and centrifuged. Two microliters from the organic layer was injected onto the GLC column.

Base-catalyzed transmethylation with sodium methylate (NaOMe) and TBME at room temperature: 50 mg of heated FAME or TAG was accurately weighed into a 5-mL screw-capped centrifuge tube and dissolved in 1 mL of the standard solution in TBME. Five-tenths milliliter of 0.2 N solution of sodium methylate in methanol was added, and the vial was closed, shaken for 1 min, and left for 2 min at room temperature. One-tenth milliliter of a 1 N solution of H_2SO_4 in water was then added and the mixture was shaken for a few seconds. The mixture was diluted with 1.5 mL of H_2O, shaken for 10 s, and centrifuged. Two microliters from the organic layer was injected directly onto the GLC column (21).

FA methylation using diazomethane: After base-catalyzed methylation with NaOMe, 0.2 mL of the heated TAG samples was evaporated under reduced pressure, redissolved in diethyl ether, and methylated by using diazomethane in a second step. A device was used (22) whereby the first tube was half-filled with diethyl ether (approximately 5 mL) and the second tube contained 3 mL of 2-(2-ethoxyethoxy)ethanol, 3 mL of diethyl ether, and 3 mL of 60% NaOH solution. Approximately 1 g of *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide was added to the second tube, and both tubes were connected. A stream of nitrogen (5–10 mL/min) was applied to the first tube, entered the second tube being saturated with ether, and carried the diazomethane generated into the vial containing the sample diluted in diethyl ether. Methylation of FA was judged to be completed upon development of a light yellow color. Two microliters was injected onto the GLC column.

GLC. FAME were analyzed by GLC using an HP 6890 chromatograph on an HP Innowax capillary column: 30 m × 0.25 mm i.d., film thickness 0.25 μm (Hewlett-Packard) under the following temperature program: 90°C (2 min), 4°C/min; 240°C (25 min). Samples were introduced into the column *via* a split injector (split ratio 1:40) at 250°C, and the flow rate of hydrogen, used as carrier gas, was 1 mL/min. Temperature of both the split injector and the FID was 250°C.

GLC–MS. GLC–MS analyses were performed with a Finnigan MAT 95 double-focusing mass spectrometer (Finnigan, Bremen, Germany) operating in the electron ionization mode. Electron energy was 70 eV, multiplier voltage 1500 V, source temperature 200°C, and transfer line 250°C. Spectral data were acquired over a mass range of 28–600 amu at a scan rate of 1 s/scan. Chromatographic conditions were the same as those used for GLC analyses.

RESULTS AND DISCUSSION

Heated samples were initially characterized by determining the total amount of the new compounds formed and their distribution in the main groups of constituents, i.e., polymeric compounds and oxidized compounds, by means of adsorption and size exclusion chromatography. Tables 1 and 2 summarize the results obtained for FAME and TAG model systems, respectively. As expected, levels of total polar compounds were much higher in TAG model systems than those found in FAME model systems. In fact, polar compounds in FAME systems included exclusively modified FAME, whereas polar compounds in TAG model systems consisted of TAG containing one to three modified fatty acyl groups; therefore, a considerable number of unoxidized fatty acyl groups were included.

As linoleic and oleic acids are the most representative FA undergoing degradation in the TAG of oils and fats, results obtained on TAG model systems are of particular interest. As can be observed in Table 2, only the samples heated for 5 h had polar compound levels lower than 25%, compatible with the

TABLE 1
Total Polar FAME (wt% on heated sample), and Their Distribution (mg/g of heated sample) in Model Systems of Methyl Oleate (MO) and Methyl Linoleate (ML) Heated at 180°C for 5, 10, and 15 h[a]

		Polar compounds		
			Distribution	
Sample	Heating period (h)	Total	FAP + FAD	OxFAM
MO	5	8.9	63	26
	10	17.8	116	67
	15	23.6	148	87
ML	5	10.0	68	32
	10	17.2	124	48
	15	26.2	193	69

[a]Values are means of duplicate analyses. FAP, FA polymers; FAD, FA dimers; OxFAM, oxidized FA monomers.

TABLE 2
Total Polar Compounds (wt% on heated sample), and Their Distribution (mg/g of heated sample) in Model Systems of Triolein (OOO) and Trilinolein (LLL) Heated at 180°C for 5, 10, and 15 h[a]

		Polar compounds		
			Distribution	
Sample	Heating period (h)	Total	TGP + TGD	OxTGM
OOO	5	20.2	95	100
	10	32.8	158	160
	15	42.7	210	207
LLL	5	21.7	156	76
	10	41.5	285	129
	15	53.2	378	152

[a]Values are means of duplicate analyses. TGP, triacylglycerol polymers; TGD, triacylglycerol dimers; OxTGM, oxidized triacylglycerol monomers.

official regulations on frying fats and oils established in many European countries (23). However, it is not unusual to find used frying oils and fats with polar compound percentages as high as those found for LLL heated for 15 h (24). In the case of TAG model systems, separation by exclusion chromatography of polar compounds also allows quantitation of DAG and FA formed through hydrolytic or thermolytic reactions. However, under the conditions used, no formation of these two groups of compounds was observed.

In spite of the differences in the degree of unsaturation of the main constituents of thermoxidized substrates, i.e., oleic and linoleic acids, the levels of polar compounds in heated OOO and LLL samples were similar after heating for 5 h (20.2 and 21.7%, respectively), as found previously in frying oils of distinct degrees of unsaturation (25–27). However, the amounts of oxidized monomers were higher in OOO samples than those obtained for the same heating period for LLL, reflecting the lower tendency toward polymerization of the less unsaturated substrate.

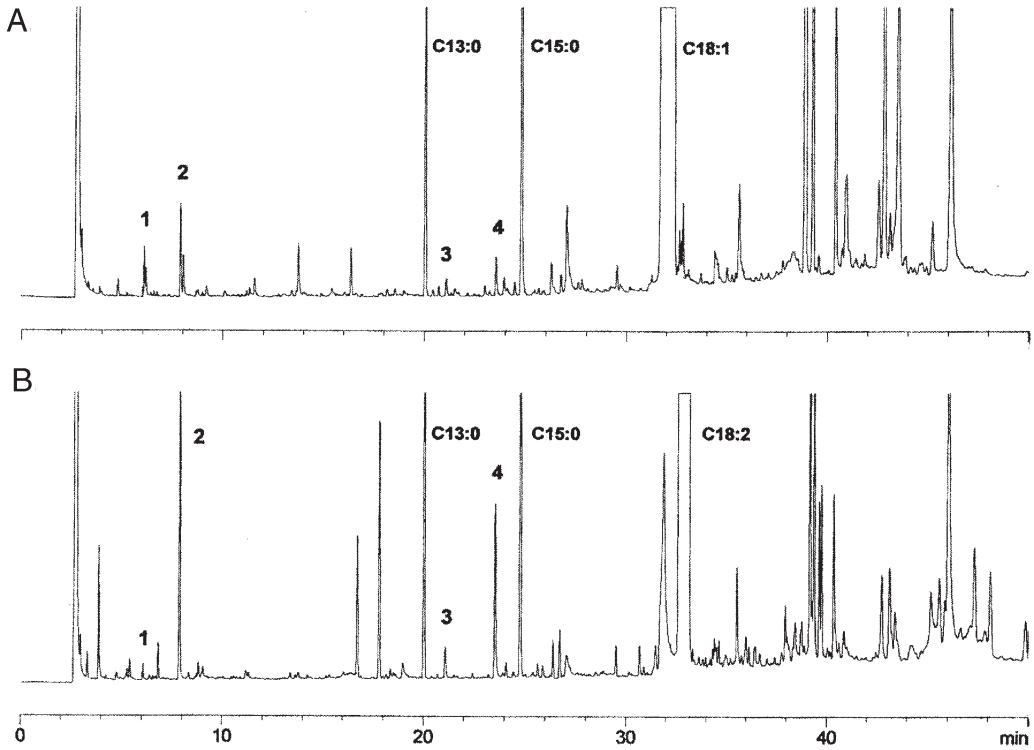

FIG. 1. Gas chromatograms of thermoxidized methyl oleate (A) and thermoxidized methyl linoleate (B). Conditions: HP Innowax capillary column (30 m × 0.25 mm i.d.; Hewlett-Packard, Avondale, PA). Temperature program: 90°C (2 min), 4°C/min; 240°C (25 min). Peak assignments: C13:0, methyl tridecanoate, internal standard (Rt = 20.0 min); C15:0, methyl pentadecanoate, internal standard (Rt = 24.8 min); C18:1, methyl oleate (Rt = 32.4 min); C18:2, methyl linoleate (Rt = 33.2 min); **1**, methyl heptanoate (Rt = 6.0 min); **2**, methyl octanoate (Rt = 7.9 min); **3**, methyl 8-oxooctanoate (Rt = 21.1 min); **4**, methyl 9-oxononanoate (Rt = 23.5 min).

Figure 1 shows representative gas chromatograms of thermoxidized MO and ML, respectively, after heating for 15 h at 180°C. Methyl C13:0 and C15:0 were added as internal standards for quantitative purposes. All peaks eluting at retention times shorter than that for the intact MO (32.0 min) or ML (32.9 min) corresponded to oxidation compounds of lower molecular mass. Among the most relevant compounds identified were saturated short-chain FAME, methyl heptanoate (C7:0, **1**) and octanoate (C8:0, **2**), and saturated short-chain aldehydic FAME, methyl 8-oxooctanoate (8-oxo-8:0, **3**) and 9-oxononanoate (9-oxo-9:0, **4**). For those compounds of molecular mass higher than that of the starting MO or ML, a complex mixture of degradation products eluted at retention times beyond those for MO or ML, where the main groups identified corresponded to epoxyacids, ketoacids, and hydroxyacids. Epoxyacids, the major compounds among those formed at high temperature, were characterized and quantitated recently (28).

The short-chain glycerol-bound compounds expected from decomposition at high temperature of 9- and 13-hydroperoxides from LLL and of 8-, 9-, 10-, and 11-hydroperoxides from OOO are well known. Reactions involve formation of saturated and unsaturated short-chain acyl groups and aldehyde acyl groups bound to the glyceridic backbone (2,4). However, in a previous work, after methylation of thermoxidized LLL only saturated C7:0 and C8:0 FAME and C8:0 and C9:0 aldehydic FAME were found in significant amounts, which was attributed to further oxidation reactions of unsaturated compounds to yield the most stable products (17). Therefore, quantitative analysis was focused on major saturated short-chain FAME and aldehydic FAME formed from oleate, i.e., methyl C7:0, C8:0, 8-oxo-8:0 and 9-oxo-9:0, or linoleate, i.e., methyl C8:0, 8-oxo-8:0 and 9-oxo-9:0. Correction factors were 0.98 for methyl C7:0 and C8:0 and 1.80 for methyl 8-oxo-8:0 and 9-oxo-9:0 (17).

In the present work, the first experiments were carried out using ML and MO heated for 5, 10, or 15 h at 180°C by simulating the normal conditions used in frying (18). The main advantage of using methyl FAME models is that analysis by GLC can be carried out directly on the heated sample, thus overcoming possible artifact formation during transesterification.

The amounts of FAME, i.e., methyl heptanoate (C7:0), octanoate (C8:0), 8-oxooctanoate (8-oxo-8:0), and 9-oxononanoate (9-oxo-9:0) in thermoxidized ML and MO without treatment are listed in Table 3. The presence in heated ML of 8-oxo-8:0, only formed through scission of the alkoxy radical from the 8-hydroperoxide from oleate, can be explained by further oxidation and decomposition of diunsaturated structures produced by the same route, i.e., 13-oxotrideca-9,11-dienoate (17). Particularly remarkable were the unexpectedly low contents of 8-oxo-8:0 FAME as compared to those of C7:0 FAME in the case of MO and the relatively low contents of 9-oxo-9:0 FAME with respect to those of C8:0 FAME in both MO and ML, considering that β-scission of the alkoxy radical from allylic hydroperoxides clearly favors formation of aldehydes (3). These results can be explained by further oxidation of aldehydic acids to diacids as previously reported (29). Quantitation of diacids has been included as well in this publication.

Similar experiments were carried out with monoacid TAG, i.e., LLL and OOO. However, the thermoxidized LLL and OOO necessarily had to be transesterified to quantitate major oxidation short-chain glycerol-bound compounds by GLC. For that purpose, we selected two base-catalyzed transmethylation methods using 2 N methanolic KOH at room temperature or using NaOMe in methanol at room temperature, which are excellent procedures for quantitation of short-chain FA and aldehydic acids (17). Methylation procedures were applied to both FAME and TAG model systems, and the results obtained are shown in Tables 4–7. Good recovery and repeatability, and the absence of artifact formation for the compounds of interest in thermoxidized FAME model systems, were demonstrated by analyzing FAME model systems after simulated methylation. As can be observed in Tables 4 and 5, the amounts found for the different compounds were similar to those obtained before the methylation step (Table 3).

With respect to TAG model systems, regardless of the procedure used, transmethylation of the samples was complete, as no partial glycerides were detected by TLC. Quantitative results for methyl C7:0, C8:0, 8-oxo-8:0, and 9-oxo-9:0 are shown in Tables 6 and 7 for thermoxidized OOO and LLL, respectively, after application of the two catalyzed transmethylation procedures selected. The amounts obtained for OOO and LLL were of the same order as those obtained for their corresponding FAME, MO and ML, respectively.

Diacid dimethyl esters deserve special comment. They were found in small amounts in model systems of FAME and TAG after base-catalyzed transmethylation (Tables 4–7). However, they were not detected in control model systems of

TABLE 3
Concentration of Short-Chain Oxidation Compounds (mg/g of heated sample) in Model Systems of MO and ML Heated at 180°C for 5, 10, or 15 h[a]

	MO			ML		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C7:0	0.46 ± 0.03	0.66 ± 0.05	0.80 ± 0.04	—	—	—
C8:0	0.74 ± 0.04	1.18 ± 0.09	1.58 ± 0.12	2.22 ± 0.04	2.70 ± 0.05	3.33 ± 0.01
8-oxo-8:0	0.27 ± 0.04	0.39 ± 0.03	0.50 ± 0.02	0.52 ± 0.02	0.70 ± 0.03	0.95 ± 0.01
9-oxo-9:0	0.73 ± 0.05	0.83 ± 0.02	1.05 ± 0.04	1.89 ± 0.05	2.58 ± 0.08	3.13 ± 0.03

[a]Values are means ± SD (n = 3). For abbreviations see Table 1.

TABLE 4
Concentration of Short-Chain Oxidation Compounds (mg/g of heated sample) in Model Systems of MO Heated at 180°C for 5, 10, or 15 h, After Base-Catalyzed Transesterification (2N KOH or NaOMe/TBME)[a]

	KOH			NaOMe/TBME		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C7:0	0.40 ± 0.04	0.63 ± 0.04	0.79 ± 0.01	0.40 ± 0.01	0.63 ± 0.02	0.82 ± 0.04
C8:0	0.64 ± 0.01	1.18 ± 0.04	1.62 ± 0.01	0.68 ± 0.01	1.14 ± 0.03	1.62 ± 0.07
8-oxo-8:0	0.28 ± 0.02	0.39 ± 0.02	0.47 ± 0.04	0.31 ± 0.02	0.37 ± 0.01	0.48 ± 0.01
C8:0 diester	Trace	Trace	0.2 ± 0.02	Trace	Trace	Trace
9-oxo-9:0	0.62 ± 0.03	0.82 ± 0.07	0.96 ± 0.02	0.72 ± 0.06	0.86 ± 0.01	1.08 ± 0.02
C9:0 diester	Trace	0.21 ± 0.02	0.49 ± 0.02	Trace	0.13 ± 0.01	0.40 ± 0.12

[a]Values are means ± SD (n = 3). Traces < 0.1. TBME, *tert*-butylmethyl ether; for other abbreviation see Table 1.

TABLE 5
Concentration of Short-Chain Oxidation Compounds (mg/g of heated sample) in Model Systems of ML Heated at 180°C for 5, 10, or 15 h, After Base-Catalyzed Transesterification (2N KOH or NaOMe/TBME)[a]

	KOH			NaOMe/TBME		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C8:0	2.12 ± 0.13	2.70 ± 0.07	3.29 ± 0.07	2.20 ± 0.02	2.84 ± 0.01	3.35 ± 0.04
8-oxo-8:0	0.46 ± 0.06	0.67 ± 0.02	0.85 ± 0.08	0.52 ± 0.02	0.69 ± 0.01	0.93 ± 0.06
C8:0 diester	Trace	0.15 ± 0.01	0.32 ± 0.03	Trace	0.12 ± 0.01	0.20 ± 0.03
9-oxo-9:0	1.74 ± 0.10	2.44 ± 0.04	2.81 ± 0.22	1.8 ± 0.05	2.46 ± 0.09	3.04 ± 0.08
C9:0 diester	0.40 ± 0.07	0.75 ± 0.06	1.10 ± 0.17	0.25 ± 0.02	0.53 ± 0.03	0.67 ± 0.03

[a]Values are means ± SD (n = 3). Traces < 0.1. For abbreviations see Tables 1 and 4.

TABLE 6
Concentration of Short-Chain Oxidation Compounds (mg/g of heated sample) in Model Systems of OOO Heated at 180°C for 5, 10, or 15 h, After Base-Catalyzed Transesterification (2N KOH or NaOMe/TBME)[a]

	KOH			NaOMe/TBME		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C7:0	0.35 ± 0.02	0.63 ± 0.03	0.92 ± 0.04	0.35 ± 0.01	0.63 ± 0.02	0.93 ± 0.05
C8:0	0.50 ± 0.05	0.88 ± 0.04	1.30 ± 0.05	0.48 ± 0.01	0.88 ± 0.02	1.31 ± 0.07
8-oxo-8:0	0.32 ± 0.03	0.49 ± 0.02	0.72 ± 0.05	0.31 ± 0.02	0.55 ± 0.04	0.83 ± 0.03
C8:0 diester	Trace	Trace	Trace	Trace	Trace	Trace
9-oxo-9:0	0.71 ± 0.06	1.08 ± 0.03	1.45 ± 0.14	0.82 ± 0.1	1.23 ± 0.06	1.67 ± 0.03
C9:0 diester	Trace	0.09 ± 0.01	0.20 ± 0.01	Trace	0.08 ± 0.01	0.19 ± 0.01

[a]Values are means ± SD (n = 3). Traces < 0.1. For abbreviations see Tables 2 and 4.

TABLE 7
Concentration of Short-Chain Oxidation Compounds (mg/g of heated sample) in Model Systems of LLL Heated at 180°C for 5, 10, or 15 h, After Base-Catalyzed Transesterification (2N KOH or NaOMe/TBME)[a]

	KOH			NaOMe/TBME		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C8:0	1.52 ± 0.02	2.44 ± 0.05	3.43 ± 0.04	1.55 ± 0.02	2.60 ± 0.01	3.40 ± 0.09
8-oxo-8:0	0.22 ± 0.01	0.36 ± 0.01	0.55 ± 0.02	0.25 ± 0.05	0.44 ± 0.02	0.65 ± 0.04
C8:0 diester	Trace	Trace	0.10 ± 0.01	Trace	Trace	0.10 ± 0.01
9-oxo-9:0	1.20 ± 0.04	1.86 ± 0.03	2.40 ± 0.07	1.21 ± 0.01	2.13 ± 0.03	2.50 ± 0.12
C9:0 diester	0.10 ± 0.02	0.41 ± 0.05	0.56 ± 0.08	0.06 ± 0.01	0.25 ± 0.04	0.41 ± 0.04

[a]Values are means ± SD (n = 3). Traces < 0.1. For abbreviations see Tables 2 and 4.

FAME before transmethylation (Table 3). A correction factor for azelaic acid dimethyl ester was calculated against the internal standards selected, C13:0 and C15:0, and the value found (1.53) was applied for quantitation. A similar weight response was assumed for suberic acid dimethyl ester.

In a previous paper, we demonstrated that diesters were not artifacts formed during transmethylation but were compounds produced by further oxidation of aldehydes during heating at high temperatures (17). However, base-catalyzed methods could only transmethylate the esterified carboxylic acid group. In fact, the presence of dimethyl esters after base-catalyzed transmethylation in FAME and TAG model systems is a clear indication of a further esterification reaction of the free carboxylic groups during thermoxidation. Such reactions

have already been reported to occur during heat treatment. Formation of estolides through esterification between a hydroxyl group and a carboxylic group is one of the major reactions occurring during the heat treatment of FA (30–32). Therefore, not only is the presence of diesters after base-catalyzed transmethylation justified, but this also suggests indirectly that quantitation of this group of compounds was incomplete because of the incapability of detecting diacid monoesterified compounds by GLC after methylation with base-catalyzed reagents.

Demonstration of the presence of monoesterified C8:0 and C9:0 diacids was achieved in two independent ways. On the one hand, the peaks corresponding to the diesters of suberic and azelaic acids showed an increase after acid methylation. Figure 2 shows representative parts of the chromatograms obtained before and after methylation with diazomethane of ML thermoxidized for 15 h, where the appearance of two new peaks, with retention times of 23.0 and 25.2 min, can be easily observed. On the other hand, monoesterified azelaic acid

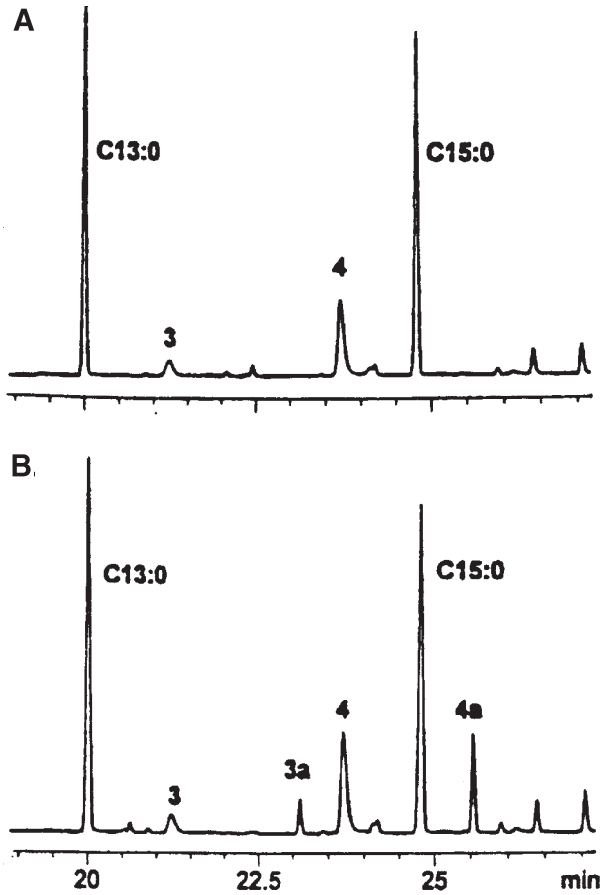

FIG. 2. Partial gas chromatograms corresponding to thermoxidized methyl linoleate before (A) and after (B) methylation with diazomethane. Conditions: HP Innowax capillary column (30 m × 0.25 mm i.d.; Hewlett-Packard, Avondale, PA). Temperature program: 90°C (2 min), 4°C/min; 240°C (25 min). Peak assignments: C13:0, methyl tridecanoate, internal standard (Rt = 20.0 min); C15:0, methyl pentadecanoate, internal standard (Rt = 24.8 min); **3**, methyl 8-oxooctanoate (Rt = 21.1 min); **3a**, dimethyl octanodiate (Rt = 22.0 min); **4**, methyl 9-oxononanoate (Rt = 23.5 min); **4a**, dimethyl nonanodiate (Rt = 25.2 min).

TABLE 8
Concentration of Suberic (C8:0) and Azelaic (C9:0) Dimethyl Esters (mg/g of heated sample) in Model Systems of OOO and LLL Heated at 180°C for 5, 10, or 15 h, After Two Methylation Steps (NaOMe/TBME + Diazomethane)[a]

	OOO			LLL		
FAME	5 h	10 h	15 h	5 h	10 h	15 h
C8:0 diester	0.35	0.73	1.11	0.10	0.25	0.49
C9:0 diester	0.72	1.70	2.42	0.98	1.71	2.86

[a]Values are means of duplicate analyses. For abbreviations see Tables 2 and 4.

was identified by GLC–MS in the original thermoxidized FAME. It eluted as a small peak overlapping with epoxy compounds, at a longer retention time (39.3 min) owing to its free carboxylic acid group.

Table 8 shows the total suberic dimethyl esters (C8:0 diesters) and azelaic dimethyl esters (C9:0 dimethyl esters) found in TAG model systems after submitting samples transesterified (NaOMe/TBME) to a second methylation step with diazomethane. By comparing these results with those obtained in Tables 6 and 7 for diesters and aldehydic esters, it is easily deduced that the major amount of diesters initially had a free carboxyl group and, overall, that oxidation from aldehydes to acids was an important reaction. Finally, oxidation of aldehydes to acids justifies the unexpected low contents of 8-oxo-8:0 FAME as compared to those of C7:0 FAME in the case of MO and OOO, as well as the relatively low contents of 9-oxo-9:0 FAME as compared to those of C8:0 FAME for the four models.

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REFERENCES

1. Capella, P., Les Produits de l'Évolution des Hydroperoxydes, *Rev. Fr. Corps Gras 36*:313–323 (1989).
2. Kamal-Eldin, A., and L.A. Appelqvist, Aldehydic Acids in Frying Oils: Formation, Toxicological Significance, and Analysis, *Grasas Aceites 47*:342–348 (1996).
3. Márquez-Ruiz, G., and M.C. Dobarganes, Short-Chain Fatty Acid Formation During Thermoxidation and Frying, *J. Sci. Food Agric. 7*:120–126 (1996).
4. Frankel, E.N., Hydroperoxide Decomposition, in *Lipid Oxidation*, edited by E.N. Frankel, The Oily Press, Dundee, 1998, pp. 55–77.
5. Márquez-Ruiz, G., M.C. Pérez-Camino, and M.C. Dobarganes, Digestibility of Fatty Acid Monomers, Dimers, and Polymers in the Rat, *J. Am Oil Chem. Soc. 69*: 930–934 (1992).
6. Márquez-Ruiz, G., M.C. Pérez-Camino, and M.C. Dobarganes, Evaluation of Hydrolysis and Absorption of Thermally Oxidized Olive Oil in Non-absorbed Lipids in the Rat, *Ann. Nutr. Metab. 37*:121–128 (1993).
7. Márquez-Ruiz, G., and M.C. Dobarganes, Assessments on the Digestibility of Oxidized Compounds from [1-^{14}C]-Linoleic

Acid Using a Combination of Chromatographic Techniques. *J. Chromatogr. 675*:1–8 (1995).
8. Kamido, H., A. Kuksis, L. Murai, J.J. Myher, and H. Pang, Preparation, Chromatography and Mass Spectrometry of Cholesteryl Ester and Glycerolipid-Bound Aldehydes, *Lipids 27*: 645–650 (1992).
9. Kamido, H., A. Kuksis, L. Marai, and J.J. Myher, Identification of Core Aldehydes Among *in vitro* Peroxidation Products of Cholesteryl Esters, *Lipids 28*:331–336 (1993).
10. Kamido, H., A. Kuksis, L. Marai, and J.J. Myher, Lipid Ester-Bound Aldehydes Among Copper-Catalyzed Peroxidation Products of Human Plasma Lipoproteins, *J. Lipid Res. 36*:1876–1886 (1995).
11. Ravandi, A., A. Kuksis, J.J. Myher, and L. Marai, Determination of Lipid Ester Ozonides and Core Aldehydes by High-Performance Liquid Chromatography with On-Line Mass Spectrometry, *J. Biochem. Biophys. Methods 30*:271–285 (1995).
12. Kuksis, A., Core Aldehydes—No Longer Neglected, *inform 11*: 746–752 (2000)
13. Minamoto, S., K. Kanazawa, H. Ashida, and M. Natake, Effect of Orally Administered 9-Oxononanoic Acid on Lipogenesis in Rat Liver, *Biochim. Biophys. Acta 958*:199–204 (1988).
14. Minamoto, S., K. Kanazawa, H. Ashida, G. Danno, and M. Natake, The Induction of Lipid Peroxidation in Rat Liver by Oral Intake of 9-Oxononanoic Acid Contained in Autoxidation Linoleic Acid, *Agric. Biol. Chem. 49*:2747–2751 (1985).
15. Kanazawa, K., and H. Ashida, Target Enzymes on Hepatic Dysfunction Caused by Dietary Products of Lipid Peroxidation, *Arch. Biochem. Biophys. 288*: 71–78 (1991).
16. Kamal-Eldin, A., G. Márquez-Ruiz, M.C. Dobarganes, and L.A. Appelqvist, Characterisation of Aldehydic Acids in Used and Unused Frying Oils, *J. Chromatogr. 776*:245–254 (1997).
17. Berdeaux, O., G. Márquez-Ruiz, and M.C. Dobarganes, Selection of Methylation Procedures for Quantitation of Short-Chain Triacylglycerol-Bound Compounds Formed During Thermoxidation, *J. Chromatogr. A 863*:171–181 (1999).
18. Barrera-Arellano, D., G. Márquez-Ruiz, and M.C. Dobarganes, A Simple Procedure to Evaluate the Performance of Fats and Oils at Frying Temperatures, *Grasas Aceites 48*:231–235 (1997).
19. Dobarganes, M.C., M.C. Pérez-Camino, and G. Márquez-Ruiz, High Performance Size Exclusion Chromatography of Polar Compounds in Heated and Non-heated Fats, *Fat Sci. Technol. 90*:308–311 (1988).
20. Dobarganes, M.C., M.C. Pérez-Camino, and R. Gutiérrez, Métodos Analíticos de Aplicación en Grasas Calentadas. I. Determinación de Ésteres Metílicos No Alterados, *Grasas Aceites 35*:172–177 (1984).
21. Cecchi, G., S. Biasini, and J. Castano, Méthanolyse Rapide des Huiles en Solvant, *Rev. Fr. Corps Gras 32*:163–164 (1985).
22. Cohen, J.D., Convenient Apparatus for the Generation of Small Amounts of Diazomethane, *J. Chromatogr. 303*:193–196 (1984).
23. Firestone, D., Worldwide Regulation of Frying Fats and Oils, *inform 4*:1366–1371.
24. Dobarganes, M.C., and G. Márquez Ruiz, Regulation of Used Frying Fats and Validity of Quick Tests for Discarding the Fats, *Grasas Aceites 49*:331–335 (1998).
25. Márquez-Ruiz, G., M. Tasioula-Margari, and M.C. Dobarganes, Quantitation and Distribution of Altered Fatty Acids in Frying Fats, *J. Am. Oil Chem. Soc. 72*:1171–1176 (1995).
26. Warner, K., P. Orr, and M. Glynn, Effect of Fatty Acid Composition of Oils on Flavor and Stability of Fried Foods, *Ibid. 74*: 347–356 (1997).
27. Warner, K., and S. Knowlton, Frying Quality and Oxidative Stability of High-Oleic Corn Oils, *Ibid. 74*: 1317–1322 (1997).
28. Berdeaux, O., G. Márquez-Ruiz, and M.C. Dobarganes, Characterization, Quantitation, and Evolution of Monoepoxy Compounds Formed in Model Systems of Fatty Acid Methyl Esters and Monoacid Triglycerides Heated at High Temperature, *Grasas Aceites 50*:53–59 (1999).
29. Passi, S., M. Picardo, C. De Luca, M. Nazzaro-Porro, L. Rossi, and G. Rotilio, Saturated Dicarboxylic Acids as Products of Unsaturated Fatty Acid Oxidation, *Biochim. Biophys. Acta 1168*: 190–198 (1993).
30. Graille, J., and M. Naudet, Mise en Evidence, Dosage et Essais d'Identification des Produits d'Altération Thermooxydative dans les Corps Gras, *Ann. Fals. Exp. Chim. 69*:179–187 (1976).
31. Ottaviani, P., J. Graille, P. Perfetti, and M. Naudet, Produits d'Altération Thermooxydative des Huiles Chauffées. II: Composés Apolaires ou Faiblement Polaires, *Chem. Phys. Lipids 24*: 57–77 (1979).
32. Perrin, J.-L., P. Perfetti, and M. Naudet, Etude Analytique Appronfondie d'Huiles Chauffées: II. Etude Comparative de Corps Gras Différents Amenés à des États d'Altération Comparables, *Rev. Fr. Corps Gras 32*:205–214 (1985).

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